

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference P23667A/RMC	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
International application No. PCT/GB 00/ 01089	International filing date (day/month/year) 29/03/2000	(Earliest) Priority Date (day/month/year) 29/03/1999
Applicant UNIVERSITY OF ULSTER et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the language, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of the sequence listing :

contained in the international application in written form.

filed together with the international application in computer readable form.

furnished subsequently to this Authority in written form.

furnished subsequently to this Authority in computer readable form.

the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. Certain claims were found unsearchable (See Box I).

3. Unity of Invention is lacking (see Box II).

4. With regard to the title,

the text is approved as submitted by the applicant.

the text has been established by this Authority to read as follows:

ANALOGS OF GASTRIC INHIBITORY PEPTIDE AND THEIR USE FOR TREATMENT OF DIABETES

5. With regard to the abstract,

the text is approved as submitted by the applicant.

the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the drawings to be published with the abstract is Figure No.

as suggested by the applicant.

because the applicant failed to suggest a figure.

because this figure better characterizes the invention.

None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No

P 00/01089

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/575 A61K38/22 A61P5/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 196 16 486 A (HANS KNOELL INST FUER NATURSTO) 30 October 1997 (1997-10-30) claims; examples ---	1,7-11
A	EP 0 869 135 A (LILLY CO ELI) 7 October 1998 (1998-10-07) page 3, line 2 - line 49; claims; examples --- -/-	1,7-11



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

& document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

1 November 2000

15/11/2000

Name and mailing address of the ISA

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Authorized officer

Fuhr, C

PATENT COOPERATION TREATY
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REC'D 18 JUL 2001
WIPO PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Article 36 and Rule 70) 14

Applicant's or agent's file reference P23667A/CPA/RMC	FOR FURTHER ACTION		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/GB00/01089	International filing date (day/month/year) 29/03/2000	Priority date (day/month/year) 29/03/1999	
International Patent Classification (IPC) or national classification and IPC C07K14/575			

Applicant

UNIVERSITY OF ULSTER et al.

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 21/09/2000	Date of completion of this report 16.07.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Halle, F Telephone No. +49 89 2399 8537



**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/01089

I. Basis of the report

1. With regard to the elements of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

Description, pages:

1-32 as originally filed

Claims, No.:

1-11 as originally filed

Drawings, sheets:

1/32-32/32 as originally filed

Sequence listing part of the description, pages:

1,2, filed with the letter of 06.07.00

2. With regard to the language, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/GB00/01089

the description, pages:
 the claims, Nos.:
 the drawings, sheets:

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):
(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;
citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	1-11
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-11
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-11
	No:	Claims	

**2. Citations and explanations
see separate sheet**

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB00/01089

In this report, it is referred to the following document:

D1: DE-A-196 16 486

The known Gastric Inhibitory Polypeptide (GIP) contains 1 to 42 amino acid residues (GIP (1-42)) and acts as an insulin-releasing hormone. This hormone can be inactivated by enzymatic degradation by a dipeptidyl peptidase-IV (DPP-IV). The present invention provides peptide analogues of GIP which are DPP-IV resistant and can therefore be used in the treatment of diabetes.

The subject-matter of claims 1-11 which is directed to said peptide analogues of GIP, to the use thereof, pharmaceutical compositions and methods, appears to be novel and to involve an inventive step (Article 33(2)(3) PCT) since the prior art does not disclose peptide GIPs modified in particular in the N-terminal amino acid residues at positions 1-3 as presently claimed. Moreover, as also indicated by the Applicant, it was not established in 1999 that DPPIV is a major inactivator of GIP in vivo, nor was it established that the N-terminal amino acid portion of GIP is a key for hormone activity.

The above comments are based on the assumption that all claims enjoy priority rights from the filing date of the priority document. In that case the document cited as P,X-document in the international search report is not considered as prior art.

The prior art document D1 is not mentioned in the description, cf. Rule 5.1(a)(ii) PCT. Moreover, a document reflecting the prior art described on pages 1-4 is not identified in the description (Rule 5.1(a)(ii) PCT).

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L SEARCH REPORTInternat
ional Application No
PCT/GB 00/01089A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/575 A61K38/22 A61P5/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, EPO-Internal, WPI Data, PAJ, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE 196 16 486 A (HANS KNOELL INST FUER NATURST0) 30 October 1997 (1997-10-30) claims; examples ---	1,7-11--
A	EP 0 869 135 A (LILLY CO ELI) 7 October 1998 (1998-10-07) page 3, line 2 - line 49; claims; examples ---	1,7-11 -/-

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *&* document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
1 November 2000	15/11/2000
Name and mailing address of the ISA European Patent Office, P.B. 5818 Patendaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Authorized officer Fuhr, C

INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/GB 00/01089

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>CHEMICAL ABSTRACTS, vol. 131, no. 1, 5 July 1999 (1999-07-05) Columbus, Ohio, US; abstract no. 944, O'HARTE, FINBARR P. M. ET AL: "NH2-terminally modified gastric inhibitory polypeptide exhibits amino- peptidase resistance and enhanced antihyperglycemic activity" XP002151675 abstract & DIABETES (4-1999), 48(4), 758-765 , 1999, -----</p>	1,7-11

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 00/01089

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
DE 19616486 A	30-10-1997	AU	721477 B	06-07-2000
		AU	3023397 A	19-11-1997
		CA	2252576 A	06-11-1997
		CN	1216468 A	12-05-1999
		WO	9740832 A	06-11-1997
		EP	0896538 A	17-02-1999
		NZ	332707 A	28-10-1999
EP 0869135 A	07-10-1998	US	5981488 A	09-11-1999

PCT

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C07K 14/575, A61K 38/22, A61P 5/48		A2	(11) International Publication Number: WO 00/58360 (43) International Publication Date: 5 October 2000 (05.10.00)
(21) International Application Number:	PCT/GB00/01089		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
(22) International Filing Date:	29 March 2000 (29.03.00)		
(30) Priority Data:	9907216.7 9917565.5	29 March 1999 (29.03.99) 27 July 1999 (27.07.99)	GB GB
(71) Applicant (for all designated States except US):	UNIVERSITY OF ULSTER [GB/GB]; Coleraine, Co Londonderry BT52 1SA (GB).		Published <i>Without international search report and to be republished upon receipt of that report.</i>
(72) Inventors; and			
(75) Inventors/Applicants (for US only):	O'HARTE, Finbarr, Paul, Mary [GB/GB]; 2 Cambourne Park, Finvoy Road, Ballymoney, Co Antrim BT53 7PG (GB). FLATT, Peter, Raymond [GB/GB]; 18 Ballymacrea Road, Portrush, Co Antrim BT56 8NR (GB).		
(74) Agent:	MURGITROYD & COMPANY; 373 Scotland Street, Glasgow G5 8QA (GB).		

(54) Title: PEPTIDE

(57) Abstract

The present invention provides peptides which stimulate the release of insulin. The peptides, based on GIP 1-42 include substitutions and/or modifications which enhance and influence secretion and/or have enhanced resistance to degradation. The invention also provides a process of N terminally modifying GIP and the use of the peptide analogues for treatment of diabetes.

FOR THE PURPOSES OF INFORMATION ONLY

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EE	Estonia						

1 **"Peptide"**

2

3 The present invention relates to the release of insulin
4 and the control of blood glucose concentration. More
5 particularly the invention relates to the use of
6 peptides to stimulate release of insulin, lowering of
7 blood glucose and pharmaceutical preparations for
8 treatment of type 2 diabetes.

9

10 Gastric inhibitory polypeptide (GIP) and glucagon-like
11 peptide-1(7-36)amide (truncated GLP-1; tGLP-1) are two
12 important insulin-releasing hormones secreted from
13 endocrine cells in the intestinal tract in response to
14 feeding. Together with autonomic nerves they play a
15 vital supporting role to the pancreatic islets in the
16 control of blood glucose homeostasis and nutrient
17 metabolism.

18

19 Dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5) has been
20 identified as a key enzyme responsible for inactivation
21 of GIP and tGLP-1 in serum. DPP IV is completely
22 inhibited in serum by the addition of diprotin A(DPA,
23 0.1 mmol/l). This occurs through the rapid removal of

1 the N-terminal dipeptides Tyr¹- Ala² and His⁷-Ala⁸
2 giving rise to the main metabolites GIP(3-42) and GLP-
3 1(9-36)amide, respectively. These truncated peptides
4 are reported to lack biological activity or to even
5 serve as antagonists at GIP or tGLP-1 receptors. The
6 resulting biological half-lives of these incretin
7 hormones *in vivo* are therefore very short, estimated to
8 be no longer than 5 min.

9
10 In situations of normal glucose regulation and
11 pancreatic B-cell sensitivity, this short duration of
12 action is advantageous in facilitating momentary
13 adjustments to homeostatic control. However, the
14 current goal of a possible therapeutic role of incretin
15 hormones, particularly tGLP-1 in NIDDM therapy is
16 frustrated by a number of factors in addition to
17 finding a convenient route of administration. Most
18 notable of these are rapid peptide degradation and
19 rapid absorption (peak concentrations reached 20 min)
20 and the resulting need for both high dosage and precise
21 timing with meals. Recent therapeutic strategies have
22 focused on precipitated preparations to delay peptide
23 absorption and inhibition of GLP-1 degradation using
24 specific inhibitors of DPP IV. A possible therapeutic
25 role is also suggested by the observation that a
26 specific inhibitor of DPP IV, isoleucine thiazolidide,
27 lowered blood glucose and enhanced insulin secretion in
28 glucose-treated diabetic obese Zucker rats presumably
29 by protecting against catabolism of the incretin
30 hormones tGLP-1 and GIP.

1 Numerous studies have indicated that tGLP-1 infusion
2 restores pancreatic B-cell sensitivity, insulin
3 secretory oscillations and improved glycemic control in
4 various groups of patients with IGT or NIDDM. Longer
5 term studies also show significant benefits of tGLP-1
6 injections in NIDDM and possibly IDDM therapy,
7 providing a major incentive to develop an orally
8 effective or long-acting tGLP-1 analogue. Several
9 attempts have been made to produce structurally
10 modified analogues of tGLP-1 which are resistant to DPP
11 IV degradation. A significant extension of serum half-
12 life is observed with His⁷- glucitol tGLP-1 and tGLP-1
13 analogues substituted at position 8 with Gly, Aib, Ser
14 or Thr. However, these structural modifications seem
15 to impair receptor binding and insulinotropic activity
16 thereby compromising part of the benefits of protection
17 from proteolytic degradation. In recent studies using
18 His⁷-glucitol tGLP-1, resistance to DPP IV and serum
19 degradation was accompanied by severe loss of insulin-
20 releasing activity.

21
22 GIP shares not only the same degradation pathway as
23 tGLP-1 but many similar physiological actions,
24 including stimulation of insulin and somatostatin
25 secretion, and the enhancement of glucose disposal.
26 These actions are viewed as key aspects in the
27 antihyperglycemic properties of tGLP-1, and there is
28 therefore good expectation that GIP may have similar
29 potential as NIDDM therapy. Indeed, compensation by
30 GIP is held to explain the modest disturbances of
31 glucose homeostasis observed in tGLP-1 knockout mice.
32 Apart from early studies, the anti-diabetic potential

1 of GIP has not been explored and tGLP-1 may seem more
2 attractive since it is viewed by some as a more potent
3 insulin secretagogue when infused at "so called"
4 physiological concentrations estimated by RIA.

5

6 The present invention aims to provide effective
7 analogues of GIP. It is one aim of the invention to
8 provide a pharmaceutical for treatment of Type 2
9 diabetes.

10

11 According to the present invention there is provided an
12 effective peptide analogue of the biologically active
13 GIP(1-42) which has improved characteristics for
14 treatment of Type 2 diabetes wherein the analogue
15 comprises at least 15 amino acid residues from the N
16 terminus of GIP(1-42) and has at least one amino acid
17 substitution or modification at position 1-3 and not
18 including Tyr¹ glucitol GIP(1-42).

19

20 The structures of human and porcine GIP(1-42) are shown
21 below. The porcine peptide differs by just two amino
22 acid substitutions at positions 18 and 34.

23

24

25 The analogue may include modification by fatty acid
26 addition at an epsilon amino group of at least one
27 lysine residue.

28

29 The invention includes Tyr¹ glucitol GIP(1-42) having
30 fatty acid addition at an epsilon amino group of at
31 least one lysine residue.

32

Fig. 1. Primary structure of human gastric inhibitory polypeptide (GIP)

1 5 10 15 20 25
NH₂-Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-His-Gln-Gln-Asp-Phe-Val-Asn-Trp
Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Asn-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-COOH
30 35 40

Fig. 2. Primary structure of porcine gastric inhibitory polypeptide (GIP)

1 5 10 15 20 25
NH₂-Tyr-Ala-Glu-Gly-Thr-Phe-Ile-Ser-Asp-Tyr-Ser-Ile-Ala-Met-Asp-Lys-Ile-Arg-Gln-Gln-Asp-Phe-Val-Asn-Trp
Leu-Leu-Ala-Gln-Lys-Gly-Lys-Lys-Ser-Asp-Trp-Lys-His-Asn-Ile-Thr-Gln-COOH
30 35 40

1 Analogues of GIP(1-42) may have an enhanced capacity to
2 stimulate insulin secretion, enhance glucose disposal,
3 delay glucose absorption or may exhibit enhanced
4 stability in plasma as compared to native GIP. They
5 also may have enhanced resistance to degradation.

6

7 Any of these properties will enhance the potency of the
8 analogue as a therapeutic agent.

9

10 Analogues having D-amino acid substitutions in the 1, 2
11 and 3 positions and/or N-glycated, N-alkylated, N-
12 acetylated or N-acylated amino acids in the 1 position
13 are resistant to degradation *in vivo*.

14

15 Various amino acid substitutions at second and third
16 amino terminal residues are included, such as GIP(1-
17 42)Gly2, GIP(1-42)Ser2, GIP(1-42)Abu2, GIP(1-42)Aib,
18 GIP(1-42)D-Ala2, GIP(1-42)Sar2, and GIP(1-42)Pro3.

19

20 Amino-terminally modified GIP analogues include N-
21 glycated GIP(1-42), N-alkylated GIP(1-42), N-acetylated

1 GIP(1-42), N-acetyl-GIP(1-42) and N-isopropyl GIP(1-
2 42).

3

4 Other stabilised analogues include those with a peptide
5 isostere bond between amino terminal residues at
6 position 2 and 3. These analogues may be resistant to
7 the plasma enzyme dipeptidyl-peptidase IV (DPP IV)
8 which is largely responsible for inactivation of GIP by
9 removal of the amino-terminal dipeptide Tyrl-Ala2.

10

11 In particular embodiments, the invention provides a
12 peptide which is more potent than human or porcine GIP
13 in moderating blood glucose excursions, said peptide
14 consisting of GIP(1-42) or N-terminal fragments of
15 GIP(1-42) consisting of up to between 15 to 30 amino
16 acid residues from the N-terminus (i.e. GIP(1-15) -
17 GIP(1-3)) with one or more modifications selected from
18 the group consisting of:

19

- 20 (a) substitution of Ala² by Gly
- 21 (b) substitution of Ala² by Ser
- 22 (c) substitution of Ala² by Abu
- 23 (d) substitution of Ala² by Aib
- 24 (e) substitution of Ala² by D-Ala
- 25 (f) substitution of Ala² by Sar
- 26 (g) substitution of Glu³ by Pro
- 27 (h) modification of Tyr¹ by acetylation
- 28 (i) modification of Tyr¹ by acylation
- 29 (j) modification of Tyr¹ by alkylation
- 30 (k) modification of Tyr¹ by glycation
- 31 (l) conversion of Ala²-Glu³ bond to a psi [CH2NH] bond

- 1 (m) conversion of Ala2-Glu3 bond to a stable peptide
- 2 isotere bond
- 3 (n) (n-isopropyl-H) 1GIP.

4

5 The invention also provides the use of Tyr¹-glucitol
6 GIP in the preparation of a medicament for the
7 treatment of diabetes.

8

9 The invention further provides improved pharmaceutical
10 compositions including analogues of GIP with improved
11 pharmacological properties.

12

13 Other possible analogues include certain commonly
14 encountered amino acids, which are not encoded by the
15 genetic code, for example, beta-alanine (beta-ala), or
16 other omega-amino acids, such as 3-amino propionic, 4-
17 amino butyric and so forth, ornithine (Orn), citrulline
18 (Cit), homoarginine (Har), t-butylalanine (t-BuA), t-
19 butylglycine (t-BuG), N-methylisoleucine (N-MeIle),
20 phenylglycine (Phg), and cyclohexylalanine (Cha),
21 norleucine (Nle), cysteic acid (Cya) and methionine
22 sulfoxide (MSO), substitution of the D form of a
23 neutral or acidic amino acid or the D form of tyrosine
24 for tyrosine.

25

26 According to the present invention there is also
27 provided a pharmaceutical composition useful in the
28 treatment of diabetes type II which comprises an
29 effective amount of the peptide as described herein, in
30 admixture with a pharmaceutically acceptable excipient.

31

1 The invention also provides a method of N-terminally
2 modifying GIP or analogues thereof the method
3 comprising the steps of synthesizing the peptide from
4 the C terminal to the penultimate N terminal amino
5 acid, adding tyrosine to a bubbler system as a F-moc
6 protected Tyr(tBu)-Wang resin, deprotecting the N-
7 terminus of the tyrosine and reacting with the
8 modifying agent, allowing the reaction to proceed to
9 completion, cleaving the modified tyrosine from the
10 Wang resin and adding the modified tyrosine to the
11 peptide synthesis reaction.

12

13 Preferably the agent is glucose, acetic anhydride or
14 pyroglutamic acid.

15

16 The invention will now be demonstrated with reference
17 to the following non-limiting example and the
18 accompanying figures wherein:

19

20 Figure 1a illustrates degradation of GIP by DPP IV.

21

22 Figure 1b illustrates degradation of GIP and Tyr¹
23 glucitol GIP by DPP IV.

24

25 Figure 2a illustrates degradation of GIP human plasma.

26

27 Figure 2b illustrates degradation of GIP and Tyr¹-
28 glucitol GIP by human plasma.

29

30

1 Figure 3 illustrates electrospray ionization mass
2 spectrometry of GIP, Tyr¹-glucitol GIP and the major
3 degradation fragment GIP(3-42).

4

5 Figure 4 shows the effects of GIP and glycated GIP on
6 plasma glucose homeostasis.

7

8 Figure 5 shows effects of GIP on plasma insulin
9 responses.

10

11 Figure 6 illustrates DPP-IV degradation of GIP 1-42.

12

13 Figure 7 illustrates DPP-IV degradation of GIP (Abu²).

14

15 Figure 8 illustrates DPP-IV degradation of GIP (Sar²).

16

17 Figure 9 illustrates DPP-IV degradation of GIP (Ser²),

18

19 Figure 10 illustrates DPP-IV degradation of N-Acetyl-
20 GIP.

21

22 Figure 11 illustrates DPP-IV degradation of glycated
23 GIP.

24

25 Figure 12 illustrates human plasma degradation of GIP.

26

27 Figure 13 illustrates human plasma degradation of GIP
28 (Abu²).

29

30 Figure 14 illustrates human plasma degradation of GIP
31 (Sar²).

32

1 Figure 15 illustrates human plasma degradation of GIP
2 (Ser²).

3

4 Figure 16 illustrates human plasma degradation of
5 glycated GIP.

6

7 Figure 17 shows the effects of various concentrations
8 of GIP 1-42 and GIP (Abu²) on insulin release from
9 BRIN-BD11 cells incubated at 5.6mM glucose.

10

11 Figure 18 shows the effects of various concentrations
12 of GIP 1-42 and GIP (Abu²) on insulin release from
13 BRIN-BD11 cells incubated at 16.7mM glucose.

14

15 Figure 19 shows the effects of various concentrations
16 of GIP 1-42 and GIP (Sar²) on insulin release from
17 BRIN-BD11 cells incubated at 5.6mM glucose.

18

19 Figure 20 shows the effects of various concentrations
20 of GIP 1-42 and GIP (Sar²) on insulin release from
21 BRIN-BD11 cells incubated at 16.7mM glucose.

22

23 Figure 21 shows the effects of various concentrations
24 of GIP 1-42 and GIP (Ser²) on insulin release from
25 BRIN-BD11 cells incubated at 5.6mM glucose.

26

27 Figure 22 shows the effects of various concentrations
28 of GIP 1-42 and GIP (Ser²) on insulin release from
29 BRIN-BD11 cells incubated at 16.7mM glucose.

30

1 Figure 23 shows the effects of various concentrations
2 of GIP 1-42 and N-Acetyl-GIP 1-42 on insulin release
3 from BRIN-BD11 cells incubated at 5.6mM glucose.

4

5 Figure 24 shows the effects of various concentrations
6 of GIP 1-42 and N-Acetyl-GIP 1-42 on insulin release
7 from BRIN-BD11 cells incubated at 16.7mM glucose.

8

9 Figure 25 shows the effects of various concentrations
10 of GIP 1-42 and glycated GIP 1-42 on insulin release
11 from BRIN-BD11 cells incubated at 5.6mM glucose.

12

13 Figure 26 shows the effects of various concentrations
14 of GIP 1-42 and glycated GIP 1-42 on insulin release
15 from BRIN-BD11 cells incubated at 16.7mM glucose.

16

17 Figure 27 shows the effects of various concentrations
18 of GIP 1-42 and GIP (Gly²) on insulin release from
19 BRIN-BD11 cells incubated at 5.6mM glucose.

20

21 Figure 28 shows the effects of various concentrations
22 of GIP 1-42 and GIP (Gly²) on insulin release from
23 BRIN-BD11 cells incubated at 16.7mM glucose.

24

25 Figure 29 shows the effects of various concentrations
26 of GIP 1-42 and GIP (Pro³) on insulin release from
27 BRIN-BD11 cells incubated at 5.6mM glucose.

28

29 Figure 30 shows the effects of various concentrations
30 of GIP 1-42 and GIP (Pro³) on insulin release from
31 BRIN-BD11 cells incubated at 16.7mM glucose.

32

1 **Example 1**

2

3 Preparation of N-terminally modified GIP and analogues
4 thereof.

5

6 The N-terminal modification of GIP is essentially a
7 three step process. Firstly, GIP is synthesised from
8 its C-terminal (starting from a Fmoc-Gln (Trt)-Wang
9 resin, Novabiochem) up to the penultimate N-terminal
10 amino-acid (Ala2) on an automated peptide synthesizer
11 (Applied Biosystems, CA, USA). The synthesis follows
12 standard Fmoc peptide chemistry protocols. Secondly,
13 the N-terminal amino acid of native GIP (Tyr) is added
14 to a manual bubbler system as a Fmoc-protected
15 Tyr(tBu)-Wang resin. This amino acid is deprotected at
16 its N-terminus (piperidine in DMF (20% v/v)) and
17 allowed to react with a high concentration of glucose
18 (glycation, under reducing conditions with sodium
19 cyanoborohydride), acetic anhydride (acetylation),
20 pyroglutamic acid (pyroglutamyl) etc. for up to 24 h as
21 necessary to allow the reaction to go to completion.
22 The completeness of reaction will be monitored using
23 the ninhydrin test which will determine the presence of
24 available free a-amino groups. Thirdly, (once the
25 reaction is complete) the now structurally modified Tyr
26 is cleaved from the wang resin (95% TFA, and 5% of the
27 appropriate scavengers. N.B. Tyr is considered to be a
28 problematic amino acid and may need special
29 consideration) and the required amount of N-terminally
30 modified-Tyr consequently added directly to the
31 automated peptide synthesiser, which will carry on the
32 synthesis, therby stitching the N-terminally modified-

1 Tyr to the α -amino of GIP(Ala2), completing the
2 synthesis of the GIP analogue. This peptide is cleaved
3 off the Wang resin (as above) and then worked up using
4 the standard Buchner filtering, precipitation, rotary
5 evaporation and drying techniques.

6

7

8

9 **Example 2**

10

11 The following example investigates preparation of Tyr^1 -
12 glycitol GIP together with evaluation of its
13 antihyperglycemic and insulin-releasing properties in
14 vivo. The results clearly demonstrate that this novel
15 GIP analogue exhibits a substantial resistance to
16 aminopeptidase degradation and increased glucose
17 lowering activity compared with the native GIP.

18

19 **Research Design and Methods**

20

21 **Materials.** Human GIP was purchased from the American
22 Peptide Company (Sunnyvale, CA, USA). HPLC grade
23 acetonitrile was obtained from Rathburn (Walkersburn,
24 Scotland). Sequencing grade trifluoroacetic acid (TFA)
25 was obtained from Aldrich (Poole, Dorset, UK). All
26 other chemicals purchased including dextran T-70,
27 activated charcoal, sodium cyanoborohydride and bovine
28 serum albumin fraction V were from Sigma (Poole,
29 Dorset, UK). Diprotin A (DPA) was purchased from
30 Calbiochem-Novabiochem (UK) Ltd. (Beeston, Nottingham,
31 UK) and rat insulin standard for RIA was obtained from
32 Novo Industria (Copenhagen, Denmark). Reversed-phase

1 Sep-Pak cartridges (C-18) were purchased from
2 Millipore-Waters (Milford, MA, USA). All water used in
3 these experiments was purified using a Milli-Q, Water
4 Purification System (Millipore Corporation, Milford,
5 MA, USA).

6

7 **Preparation of Tyr¹-glucitol GIP.** Human GIP was
8 incubated with glucose under reducing conditions in 10
9 mmol/l sodium phosphate buffer at pH 7.4 for 24 h. The
10 reaction was stopped by addition of 0.5 mol/l acetic
11 acid (30 µl) and the mixture applied to a Vydac (C-
12 18) (4.6 x 250mm) analytical HPLC column (The
13 Separations Group, Hesperia, CA, USA) and gradient
14 elution conditions were established using aqueous/TFA
15 and acetonitrile/TFA solvents. Fractions corresponding
16 to the glycated peaks were pooled, taken to dryness
17 under vacuum using an AES 1000 Speed-Vac concentrator
18 (Life Sciences International, Runcorn, UK) and purified
19 to homogeneity on a Supelcosil (C-8) (4.6 x 150mm)
20 column (Supelco Inc., Poole, Dorset, UK).

21

22 **Degradation of GIP and Tyr¹-glucitol GIP by DPP IV.**
23 HPLC-purified GIP or Tyr¹-glucitol GIP were incubated
24 at 37°C with DPP-IV (5mU) for various time periods in a
25 reaction mixture made up to 500 µl with 50 mmol/l
26 triethanolamine-HCl, pH 7.8 (final peptide
27 concentration 1 µmol/l). Enzymatic reactions were
28 terminated after 0, 2, 4 and 12 hours by addition of 5
29 µl of 10% (v/v) TFA/water. Samples were made up to a
30 final volume of 1.0 ml with 0.12% (v/v) TFA and stored
31 at -20°C prior to HPLC analysis.

1
2 **Degradation of GIP and Tyr¹-glucitol GIP by human**
3 **plasma.** Pooled human plasma (20 µl) taken from six
4 healthy fasted human subjects was incubated at 37°C
5 with GIP or Tyr¹-glucitol GIP (10 µg) for 0 and 4 hours
6 in a reaction mixture made up to 500 µl, containing 50
7 mmol/l triethanolamine/HCL buffer pH 7.8. Incubations
8 for 4 hours were also performed in the presence of
9 diprotin A (5 mU). The reactions were terminated by
10 addition of 5 µl of TFA and the final volume adjusted
11 to 1.0 ml using 0.1% v/v TFA/water. Samples were
12 centrifuged (13,000g, 5 min) and the supernatant
13 applied to a C-18 Sep-Pak cartridge (Millipore-Waters)
14 which was previously primed and washed with 0.1% (v/v)
15 TFA/water. After washing with 20 ml 0.12% TFA/water,
16 bound material was released by elution with 2 ml of 80%
17 (v/v) acetonitrile/water and concentrated using a
18 Speed-Vac concentrator (Runcorn, UK). The volume was
19 adjusted to 1.0ml with 0.12% (v/v) TFA/water prior to
20 HPLC purification.

21
22 **HPLC analysis of degraded GIP and Tyr¹-glucitol GIP.**
23 Samples were applied to a Vydac C-18 widebore column
24 equilibrated with 0.12% (v/v) TFA/H₂O at a flow rate
25 of 1.0 ml/min. Using 0.1% (v/v) TFA in 70%
26 acetonitrile/H₂O, the concentration of acetonitrile in
27 the eluting solvent was raised from 0% to 31.5% over 15
28 min, to 38.5% over 30 min and from 38.5% to 70% over 5
29 min, using linear gradients. The absorbance was
30 monitored at 206 nm and peak areas evaluated using a

1 model 2221 LKB integrator. Samples recovered manually
2 were concentrated using a Speed-Vac concentrator.

3

4 **Electrospray ionization mass spectrometry (ESI-MS).**
5 Samples for ESI-MS analysis containing intact and
6 degradation fragments of GIP (from DPP IV and plasma
7 incubations) as well as Tyr¹-glucitol GIP, were further
8 purified by HPLC. Peptides were dissolved
9 (approximately 400 pmol) in 100 μ l of water and applied
10 to the LCQ benchtop mass spectrometer (Finnigan MAT,
11 Hemel Hempstead, UK) equipped with a microbore C-18
12 HPLC column (150 x 2.0mm, Phenomenex, UK, Ltd,
13 Macclesfield). Samples (30 μ l direct loop injection)
14 were injected at a flow rate of 0.2ml/min, under
15 isocratic conditions 35% (v/v) acetonitile/water. Mass
16 spectra were obtained from the quadripole ion trap mass
17 analyzer and recorded. Spectra were collected using
18 full ion scan mode over the mass-to-charge (m/z) range
19 150-2000. The molecular masses of GIP and related
20 structures were determined from ESI-MS profiles using
21 prominent multiple charged ions and the following
22 equation $M_r = iM_i - iM_h$ (where M_r = molecular mass; M_i =
23 m/z ratio; i = number of charges; M_h = mass of a
24 proton).

25

26 **In vivo biological activity of GIP and Try¹-glucitol**
27 **GIP.** Effects of GIP and Tyr¹-glucitol GIP on plasma
28 glucose and insulin concentrations were examined using
29 10-12 week old male Wistar rats. The animals were
30 housed individually in an air conditioned room and
31 22 \pm 2°C with a 12 hour light/12 hour dark cycle.
32 Drinking water and a standard rodent maintenance diet

1 (Trouw Nutrition, Belfast) were supplied *ad libitum*.
2 Food was withdrawn for an 18 hour period prior to
3 intraperitoneal injection of glucose alone (18mmol/kg
4 body weight) or in combination with either GIP or Tyr¹-
5 glucitol GIP (10 nmol/kg). Test solutions were
6 administered in a final volume of 8 ml/kg body weight.
7 Blood samples were collected at 0, 15, 30 and 60
8 minutes from the cut tip of the tail of conscious rats
9 into chilled fluoride/heparin microcentrifuge tubes
10 (Sarstedt, Nümbrecht, Germany). Samples were
11 centrifuged using a Beckman microcentrifuge for about
12 30 seconds at 13,000 g. Plasma samples were aliquoted
13 and stored at -20°C prior to glucose and insulin
14 determinations. All animal studies were done in
15 accordance with the Animals (Scientific Procedures) Act
16 1986.

17

18 Analyses. Plasma glucose was assayed by an automated
19 glucose oxidase procedure using a Beckman Glucose
20 Analyzer II [33]. Plasma insulin was determined by
21 dextran charcoal radioimmunoassay as described
22 previously [34]. Incremental areas under plasma
23 glucose and insulin curves (AUC) were calculated using
24 a computer program (CAREA) employing the trapezoidal
25 rule [35] with baseline subtraction. Results are
26 expressed as mean ± SEM and values were compared using
27 the Student's unpaired *t*-test. Groups of data were
28 considered to be significantly different if *P*<0.05.

29

30 **Results**

31

1 **Degradation of GIP and Tyr¹-glucitol GIP by DPP IV.**
2 Figure 1 illustrates the typical peak profiles obtained
3 from the HPLC separation of the products obtained from
4 the incubation of GIP (Fig 1a) or Tyr¹-glucitol GIP
5 (Fig 1b) with DPP IV for 0, 2, 4 and 12 hours. The
6 retention times of GIP and Tyr¹-glucitol GIP at t=0
7 were 21.93 minutes and 21.75 minutes respectively.
8 Degradation of GIP was evident after 4 hours incubation
9 (54% intact), and by 12 hours the majority (60% of
10 intact GIP was converted to the single product with a
11 retention time of 21.61 minutes. Tyr¹-glucitol GIP
12 remained almost completely intact throughout 2-12 hours
13 incubation. Separation was on a Vydac C-18 column using
14 linear gradients of 0% to 31.5% acetonitrile over 15
15 minutes, to 38.5% over 30 minutes and from 38.5 to 70%
16 acetonitrile over 5 minutes.

17
18 **Degradation of GIP and Tyr¹-glucitol GIP by human**
19 **plasma.** Figure 2 shows a set of typical HPLC profiles
20 of the products obtained from the incubation of GIP or
21 Tyr¹-glucitol GIP with human plasma for 0 and 4 h. GIP
22 (Fig 2a) with a retention time of 22.06 min was readily
23 metabolised by plasma within 4 hours incubation giving
24 rise to the appearance of a major degradation peak with
25 a retention time of 21.74 minutes. In contrast, the
26 incubation of Tyr¹-glucitol GIP under similar
27 conditions (Fig 2b) did not result in the formation of
28 any detectable degradation fragments during this time
29 with only a single peak being observed with a
30 retention time of 21.77 minutes. Addition of diprotin
31 A, a specific inhibitor of DPP IV, to GIP during the 4
32 hours incubation completely inhibited degradation of

1 the peptide by plasma. Peaks corresponding with intact
2 GIP, GIP (3-42) and Tyr¹ - glucitol GIP are indicated.
3 A major peak corresponding to the specific DPP IV
4 inhibitor tripeptide DPA appears in the bottom panels
5 with retention time of 16-29 min.

6

7 **Identification of GIP degradation fragments by ESI-MS.**
8 Figure 3 shows the monoisotopic molecular masses
9 obtained for GIP, (panel A), Tyr¹-glucitol GIP (panel
10 B) and the major plasma degradation fragment of GIP
11 (panel C) using ESI-MS. The peptides analyzed were
12 purified from plasma incubations as shown in Figure 2.
13 Peptides were dissolved (approximately 400 pmol) in
14 100 μ l of water and applied to the LC/MS equipped with a
15 microbore C-18 HPLC column. Samples (30 μ l direct loop
16 injection) were applied at a flow rate of 0.2ml/min,
17 under isocratic conditions 35% acetonitrile/water.
18 Mass spectra were recorded using a quadripole ion trap
19 mass analyzer. Spectra were collected using full ion
20 scan mode over the mass-to-charge (m/z) range 150-2000.
21 The molecular masses (M_r) of GIP and related structures
22 were determined from ESI-MS profiles using prominent
23 multiple charged ions and the following equation
24 M_r=iM_i-iM_h. The exact molecular mass (M_r) of the
25 peptides were calculated using the equation M_r = iM_i -
26 iM_h as defined in Research Design and Methods. After
27 spectral averaging was performed, prominent multiple
28 charges species (M+3H)³⁺ and (M+4H)⁴⁺ were detected from
29 GIP at m/z 1661.6 and 1246.8, corresponding to intact
30 M_r 4981.8 and 4983.2 Da, respectively (Fig. 3A).
31 Similarly, for Tyr¹-glucitol GIP ((M+4H)⁴⁺ and (M+5H)⁵⁺)
32 were detected at m/z 1287.7 and 1030.3, corresponding

1 to intact molecular masses of M^{\ddagger} 5146.8 and 5146.5 Da,
2 respectively (Fig. 3B). The difference between the
3 observed molecular masses of the quadruply charged GIP
4 and the N-terminally modified GIP species (163.6 Da)
5 indicated that the latter peptide contained a single
6 glucitol adduct corresponding to Tyr^1 -glucitol GIP.
7 Figure 3C shows the prominent multiply charged species
8 $(M+3H)^{3+}$ and $(M+4H)^{4+}$ detected from the major fragment
9 of GIP at m/z 1583.8 and 1188.1, corresponding to
10 intact M^{\ddagger} 4748.4 and 4748 Da, respectively (Figure 3C).
11 This corresponds with the theoretical mass of the N-
12 terminally truncated form of the peptide GIP(3-42).
13 This fragment was also the major degradation product of
14 DPP IV incubations (data not shown).
15

16 **Effects of GIP and Tyr^1 -glucitol GIP on plasma glucose**
17 **homeostasis.** Figures 4 and 5 show the effects of
18 intraperitoneal (ip) glucose alone (18mmol.kg) (control
19 group), and glucose in combination with GIP or Tyr^1 -
20 glucitol GIP (10nmol/kg) on plasma glucose and insulin
21 concentrations.

22
23 (4A) Plasma glucose concentrations after i.p. glucose
24 alone (18mmol/kg) (control group), or glucose in
25 combination with either GIP or Tyr^1 -glucitol GIP
26 (10nmol/kg). The time of injection is indicated by the
27 arrow (0 min). (4B) Plasma glucose AUC values for 0-60
28 min post injection. Values are mean \pm SEM for six
29 rats. ** $P<0.01$, *** $P<0.001$ compared with GIP and Tyr^1 -
30 glucitol GIP; $\dagger P<0.05$, $\ddagger P<0.01$ compared with non-
31 gluculated GIP.

1 (5A). Plasma insulin concentrates after i.p. glucose
2 along (18 mmol/kg) (control group), or glucose in
3 combination with either with GIP or glycated GIP
4 (10nmol/kg). The time of injection is indicated by the
5 arrow. (5B) Plasma insulin AUC values were calculated
6 for each of the 3 groups up to 90 minutes post
7 injection. The time of injection is indicated by the
8 arrow (0 min). Plasma insulin AUC values for 0-60 min
9 post injection. Values are mean \pm SEM for six rats.
10 *P<0.05, **P<0.001 compared with GIP and Tyr¹-glucitol
11 GIP; †P<0.05, ††P<0.01 compared with non-glycated GIP.
12

13 Compared with the control group, plasma glucose
14 concentrations and area under the curve (AUC) were
15 significantly lower following administration of either
16 GIP or Tyr¹- glucitol GIP (Figure 4A, B). Furthermore,
17 individual values at 15 and 30 minutes together with
18 AUC were significantly lower following administration
19 of Tyr¹-glucitol GIP as compared to GIP. Consistent
20 with the established insulin-releasing properties of
21 GIP, plasma insulin concentrations of both peptide-
22 treated groups were significantly raised at 15 and 30
23 minutes compared with the values after administration
24 of glucose alone (Figure 5A). The overall insulin
25 responses, estimated as AUC were also signigicantly
26 greater for the two peptide-treated groups (Figure 5B).
27 Despite lower prevailing glucose concentrations than
28 the GIP-treated group, plasma insulin response,
29 calculated as AUC, following Tyr¹-glucitol GIP was
30 significantly greater than after GIP (Figure 5B). The
31 significant elevation of plasma insulin at 30 minutes
32 is of particular note, suggesting that the insulin-

1 releasing action of Tyr¹-glucitol GIP is more
2 protracted than GIP even in the face of a diminished
3 glycemic stimulus (Figures 4A, 5A).

4

5 **Discussion**

6

7 The forty two amino acid GIP is an important incretin
8 hormone released into the circulation from endocrine K-
9 cells of the duodenum and jejunum following ingestion
10 of food. The high degree of structural conservation
11 of GIP among species supports the view that this
12 peptide plays an important role in metabolism.

13 Secretion of GIP is stimulated directly by actively
14 transported nutrients in the gut lumen without a
15 notable input from autonomic nerves. The most
16 important stimulants of GIP release are simple sugars
17 and unsaturated long chain fatty acids, with amino
18 acids exerting weaker effects. As with tGLP-1, the
19 insulin-releasing effect of GIP is strictly glucose-
20 dependent. This affords protection against
21 hypoglycemia and thereby fulfills one of the most
22 desirable features of any current or potentially new
23 antidiabetic drug.

24

25 The present results demonstrate for the first time that
26 Tyr¹-glucitol GIP displays profound resistance to serum
27 and DPP IV degradation. Using ESI-MS the present study
28 showed that native GIP was rapidly cleaved *in vitro* to
29 a major 4748.4 Da degradation product, corresponding to
30 GIP(3-42) which confirmed previous findings using
31 matrix-assisted laser desorption ionization time-of-
32 flight mass spectrometry. Serum degradation was

1 completely inhibited by diprotin A (Ile-Pro-Ile), a
2 specific competitive inhibitor of DPP IV, confirming
3 this as the main enzyme for GIP inactivation *in vivo*.
4 In contrast, Tyr¹-glucitol GIP remained almost
5 completely intact after incubation with serum or DPP IV
6 for up to 12 hours. This indicates that glycation of
7 GIP at the amino-terminal Tyr¹ residue masks the
8 potential cleavage site from DPP IV and prevents
9 removal of the Tyr¹-Ala² dipeptide from the N-terminus
10 preventing the formation of GIP(3-42).

11
12 Consistent with *in vitro* protection against DPP IV,
13 administration of Tyr¹-glucitol GIP significantly
14 enhanced the antihyperglycemic activity and
15 insulin-releasing action of the peptide when
16 administered with glucose to rats. Native GIP enhanced
17 insulin release and reduced the glycemic excursion as
18 observed in many previous studies. However, amino-
19 terminal glycation of GIP increased the insulin-
20 releasing and antihyperglycemic actions of the peptide
21 by 62% and 38% respectively, as estimated from AUC
22 measurements. Detailed kinetic analysis is difficult
23 due to necessary limitation of sampling times, but the
24 greater insulin concentrations following Tyr¹-glucitol
25 GIP as opposed to GIP at 30 minutes post-injection is
26 indicative of a longer half-life. The glycemic rise
27 was modest in both peptide-treated groups and glucose
28 concentrations following injection of Tyr¹-glucitol GIP
29 were consistently lower than after GIP. Since the
30 insulinotropic actions of GIP are glucose-dependent, it
31 is likely that the relative insulin-releasing potency

1 of Tyr¹-glucitol GIP is greatly underestimated in the
2 present *in vivo* experiments.

3

4 *In vitro* studies in the laboratory of the present
5 inventors using glucose-responsive clonal B-cells
6 showed that the insulin-releasing potency of Tyr¹-
7 glucitol GIP was several order of magnitude greater
8 than GIP and that its effectiveness was more sensitive
9 to change of glucose concentrations within the
10 physiological range. Together with the present *in vivo*
11 observations, this suggests that N-terminal glycation
12 of GIP confers resistance to DPP IV degradation whilst
13 enhancing receptor binding and insulin secretory
14 effects on the B-cell. These attributes of Tyr¹-
15 glucitol GIP are fully expressed *in vivo* where DPP IV
16 resistance impedes degradation of the peptide to GIP(3-
17 42), thereby prolonging the half-life and enhancing
18 effective concentrations of the intact biologically
19 active peptide. It is thus possible that glycated GIP
20 is enhancing insulin secretion *in vivo* both by enhanced
21 potency at the receptor as well as improving DPP IV
22 resistance. Thus numerous studies have shown that GIP
23 (3-42) and other N-terminally modified fragments,
24 including GIP(4-42), and GIP (17-42) are either weakly
25 effective or inactive in stimulating insulin release.
26 Furthermore, evidence exists that N-terminal deletions
27 of GIP result in receptor antagonist properties in GIP
28 receptor transfected Chinese hamster kidney cells [9],
29 suggesting that inhibition of GIP catabolism would also
30 reduce the possible feedback antagonism at the receptor
31 level by the truncated GIP(3-42).

32

1 In addition to its insulinotropic actions, a number of
2 other potentially important extrapancreatic actions of
3 GIP may contribute to the enhanced antihyperglycemic
4 activity and other beneficial metabolic effects of
5 Tyr¹-glucitol GIP. These include the stimulation of
6 glucose uptake in adipocytes, increased synthesis of
7 fatty acids and activation of lipoprotein lipase in
8 adipose tissue. GIP also promotes plasma triglyceride
9 clearance in response to oral fat loading. In liver,
10 GIP has been shown to enhance insulin-dependent
11 inhibition of glycogenolysis. GIP also reduces both
12 glucagon-stimulated lipolysis in adipose tissue as well
13 as hepatic glucose production. Finally, recent
14 findings indicate that GIP has a potent effect on
15 glucose uptake and metabolism in mouse isolated
16 diaphragm muscle. This latter action may be shared
17 with tGLP-1 and both peptides have additional benefits
18 of stimulating somatostatin secretion and slowing down
19 gastric emptying and nutrient absorption.

20
21 In conclusion, this study has demonstrated for the
22 first time that the glycation of GIP at the amino-
23 terminal Tyr¹ residue limits GIP catabolism through
24 impairment of the proteolytic actions of serum
25 peptidases and thus prolongs its half-life *in vivo*.
26 This effect is accompanied by enhanced
27 antihyperglycemic activity and raised insulin
28 concentrations *in vivo*, suggesting that such DPP IV
29 resistant analogues should be explored alongside tGLP-1
30 as potentially useful therapeutic agents for NIDDM.
31 Tyr¹-glucitol GIP appears to be particularly
32 interesting in this regard since such amino-terminal

1 modification of GIP enhances rather than impairs
2 glucose-dependent insulinotropic potency as was
3 observed recently for tGLP-1.

4

5 **Example 3**

6

7 This example further looked at the ability of
8 additional N-terminal structural modifications of GIP
9 in preventing inactivation by DPP and in plasma and
10 their associated increase in both the insulin-releasing
11 potency and potential therapeutic value. Native human
12 GIP, glycated GIP, acetylated GIP and a number of GIP
13 analogues with N-terminal amino acid substitutions were
14 tested.

15

16 **Materials and Methods**

17

18 **Reagents**

19

20 High-performance liquid chromatography (HPLC) grade
21 acetonitrile was obtained from Rathburn (Walkersburn,
22 Scotland). Sequencing grade trifluoroacetic acid (TFA)
23 was obtained from Aldrich (Poole, Dorset, UK).

24 Dipeptidyl peptidase IV was purchased from Sigma
25 (Poole, Dorset, UK), and Diprotin A was purchased from
26 Calbiochem Novabiochem (Beeston, Nottingham, UK). RPMI
27 1640 tissue culture medium, foetal calf serum,
28 penicillin and streptomycin were all purchased from
29 Gibco (Paisley, Strathclyde, UK). All water used in
30 these experiments was purified using a Milli-Q, Water
31 Purification System (Millipore, Millford, MA, USA).

1 All other chemicals used were of the highest purity
2 available.

3

4 **Synthesis of GIP and N-terminally modified GIP
5 analogues**

6

7 GIP, GIP(Abu2), GIP(Sar2), GIP(Ser2), GIP(Gly2) and
8 GIP(Pro3) were sequentially synthesised on an Applied
9 Biosystems automated peptide synthesizer (model 432A)
10 using standard solid-phase Fmoc procedure, starting
11 with an Fmoc-Gln-Wang resin. Following cleavage from
12 the resin by trifluoroacetic acid: water, thioanisole,
13 ethanedithiol (90/2.5/5/2.5, a total volume of 20 ml/g
14 resin), the resin was removed by filtration and the
15 filtrate volume was decreased under reduced pressure.

16 Dry diethyl ether was slowly added until a precipitate
17 was observed. The precipitate was collected by low-
18 speed centrifugation, resuspended in diethyl ether and
19 centrifuged again, this procedure being carried out at
20 least five times. The pellets were then dried in vacuo
21 and judged pure by reversed-phase HPLC on a Waters
22 Millennium 2010 chromatography system (Software version
23 2.1.5.). N-terminal glycated and acetylated GIP were
24 prepared by minor modification of a published method.

25

26 Electrospray ionization-mass spectrometry (ESI-MS) was
27 carried out as described in Example 2.

28

29 Degradation of GIP and novel GIP analogues by DPP IV
30 and human plasma was carried out as described in
31 Example 2.

32

1 Culture of insulin secreting cells

2

3 BRIN-BD11 cells [30] were cultured in sterile tissue
4 culture flasks (Corning, Glass Works, UK) using RPMI-
5 1640 tissue culture medium containing 10% (v/v) foetal
6 calf serum, 1% (v/v) antibiotics (100 U/ml penicillin,
7 0.1 mg/ml streptomycin) and 11.1 mM glucose. The cells
8 were maintained at 37°C in an atmosphere of 5% CO₂ and
9 95% air using a LEEC incubator (Laboratory Technical
10 Engineering, Nottingham, UK).

11

12 Acute tests for insulin secretion

13

14 Before experimentation, the cells were harvested from
15 the surface of the tissue culture flasks with the aid
16 of trypsin/EDTA (Gibco), seeded into 24-multiwell
17 plates (Nunc, Roskilde, Denmark) at a density of 1.5 x
18 10⁵ cells per well, and allowed to attach overnight at
19 37°C. Acute tests for insulin release were preceded by
20 40 min pre-incubation at 37°C in 1.0 ml Krebs Ringer
21 bicarbonate buffer (115 mM NaCl, 4.7 mM KCl, 1.28 mM
22 CaCl₂, 1.2 mM KH₂PO₄, 1.2 mM MgSO₄, 10 mM NaHCO₃, 5 g/l
23 bovine serum albumin, pH 7.4) supplemented with 1.1 mM
24 glucose. Test incubations were performed (n=12) at two
25 glucose concentrations (5.6 mM and 16.7 mM) with a
26 range of concentrations (10⁻¹³ to 10⁻⁸ M) of GIP or GIP
27 analogues. After 20 min incubation, the buffer was
28 removed from each well and aliquots (200 µl) were used
29 for measurement of insulin by radioimmunoassay [31].

30

31 Statistical analysis

32

1 Results are expressed as mean \pm S.E.M. and values were
2 compared using the Student's unpaired t-test. Groups
3 of data were considered to be significantly different
4 if $P < 0.05$.

5

6 **Results and Discussion**

7

8 Structural identification of GIP and GIP analogues by
9 ESI-MS

10

11 The monoisotopic molecular masses of the peptides were
12 determined using ESI-MS. After spectral averaging was
13 performed, prominent multiple charged species $(M+3H)^{3+}$
14 and $(M+4H)^{4+}$ were detected for each peptide. Calculated
15 molecular masses confirmed the structural identity of
16 synthetic GIP and each of the N-terminal analogues.

17

18 **Degradation of GIP and novel GIP analogues by DPP-IV**

19

20 Figs. 6-11 illustrate the typical peak profiles
21 obtained from the HPLC separation of the reaction
22 products obtained from the incubation of GIP,
23 GIP(Abu2), GIP(Sar2), GIP(Ser2), glycated GIP and
24 acetylated GIP with DPP IV, for 0, 2, 4, 8 and 24 h.
25 The results summarised in Table 1 indicate that
26 glycated GIP, acetylated GIP, GIP(Ser2) are GIP(Abu2)
27 more resistant than native GIP to in vitro degradation
28 with DPP IV. From these data GIP(Sar2) appears to be
29 less resistant.

30

31 **Degradation of GIP and GIP analogues by human plasma**

32

1 Figs. 12-16 show a representative set of HPLC profiles
2 obtained from the incubation of GIP and GIP analogues
3 with human plasma for 0, 2, 4, 8 and 24 h. Observations
4 were also made after incubation for 24 h in the
5 presence of DPA. These results are summarised in Table
6 2 are broadly comparable with DPP IV incubations, but
7 conditions which more closely mirror in vivo conditions
8 are less enzymatically severe. GIP was rapidly degraded
9 by plasma. In comparison, all analogues tested
10 exhibited resistance to plasma degradation, including
11 GIP(Sar2) which from DPP IV data appeared least
12 resistant of the peptides tested. DPA substantially
13 inhibited degradation of GIP and all analogues tested
14 with complete abolition of degradation in the cases of
15 GIP(Abu2), GIP(Ser2) and glycated GIP. This indicates
16 that DPP IV is a key factor in the in vivo degradation
17 of GIP.

18

19 Dose-dependent effects of GIP and novel GIP analogues
20 on insulin secretion

21

22 Figs. 17-30 show the effects of a range of
23 concentrations of GIP, GIP(Abu2), GIP(Sar2), GIP(Ser2),
24 acetylated GIP, glycated GIP, GIP(Gly2) and GIP(Pro3)
25 on insulin secretion from BRIN-BD11 cells at 5.6 and
26 16.7 mM glucose. Native GIP provoked a prominent and
27 dose-related stimulation of insulin secretion.

28 Consistent with previous studies [28], the glycated GIP
29 analogue exhibited a considerably greater
30 insulinotropic response compared with native peptide.
31 N-terminal acetylated GIP exhibited a similar pattern
32 and the GIP(Ser2) analogue also evoked a strong

1 response. From these tests, GIP(Gly2) and GIP(Pro3)
2 appeared to be the least potent analogues in terms of
3 insulin release. Other stable analogues tested, namely
4 GIP(Abu2) and GIP(Sar2), exhibited a complex pattern of
5 responsiveness dependent on glucose concentration and
6 dose employed. Thus very low concentrations were
7 extremely potent under hyperglycaemic conditions (16.7
8 mM glucose). This suggests that even these analogues
9 may prove therapeutically useful in the treatment of
10 type 2 diabetes where insulinotropic capacity combined
11 with in vivo degradation dictates peptide potency.

1 **Table 1** : % Intact peptide remaining after incubation
 2 with DPPIV

Peptide	% Intact peptide remaining after time (h)				
	0	2	4	8	24
GIP 1-42	100	52 ± 1	23 ± 1	0	0
Glycated GIP	100	100	100	100	100
GIP (Abu ²)	100	38 ± 1	28 ± 2	0	0
GIP (Ser ²)	100	77 ± 2	60 ± 1	32 ± 4	0
GIP (Sar ²)	100	28 ± 2	8	0	0
N-Acetyl-GIP	100	100	100	100	0

3 **Table 2** : % Intact peptide remaining after incubation
 4 with human plasma

Peptide	% Intact peptide remaining after incubations with human plasma					
	0	2	4	8	24	DPA
GIP 1-42	100	52 ± 1	23 ± 1	0	0	68 ± 2
Glycated GIP	100	100	100	100	100	100
GIP (Abu ²)	100	38 ± 1	28 ± 2	0	0	100
GIP (Ser ²)	100	77 ± 2	60 ± 1	32 ± 4	0	63 ± 3
GIP (Sar ²)	100	28 ± 2	8	0	0	100

5 Tables represent the percentage of intact peptide (i.e.
 6 GIP 1-42) relative to the major degradation product GIP
 7 3-42. Values were taken from HPLC traces performed in
 8 triplicate and the mean and S.E.M. values calculated.
 9 DPA is diprotin A, a specific inhibitor of DPPIV.

1 CLAIMS

2

3 1. A peptide analogue of GIP (1-42) comprising at
4 least 15 amino acid residues from the N terminal
5 end of GIP (1-42) having a least one amino acid
6 substitution or modification at position 1-3 and
7 not including Tyr¹ glucitol GIP (1-42).

8

9 2. A peptide analogue as claimed in claim 1 including
10 modification by fatty acid addition at an epsilon
11 amino group of at least one lysine residue.

12

13 3. A peptide analogue of biologically active GIP (1-
14 42) wherein the analogue is Tyr¹ glucitol GIP (1-
15 42) modified by fatty acid addition at an epsilon
16 amino group of at least one lysine residue.

17

18 4. A peptide analogue as claimed in any of the
19 preceding claims wherein the substitution or
20 modification is chosen from the group comprising
21 D-amino acid substitutions in 1, 2 and/or 3
22 positions and/or N terminal glycation, alkylation,
23 acetylation or acylation.

24

25 5. A peptide analogue as claimed in any of the
26 preceding claims wherein the amino acid in the 2
27 or 3 position is substituted by lysine, serine, 4-
28 amino butyric, Aib, D-alanine, Sarcosine or
29 Proline.

30

31 6. An analogue as claimed in any of the preceding
32 claims wherein the N terminus is modified by one

1 of the group of modifications include glycation,
2 alkylation, acetylation or by the addition of an
3 isopropyl group.

4

5 7. Use of an analogue as claimed in any of the
6 preceding claims in the preparation of a
7 medicament for the treatment of diabetes.

8

9 8. A pharmaceutical composition including an analogue
10 as claimed in any of the preceding claims.

11

12 9. A pharmaceutical composition as claimed in claim 8
13 in admixture with a pharmaceutically acceptable
14 excipient.

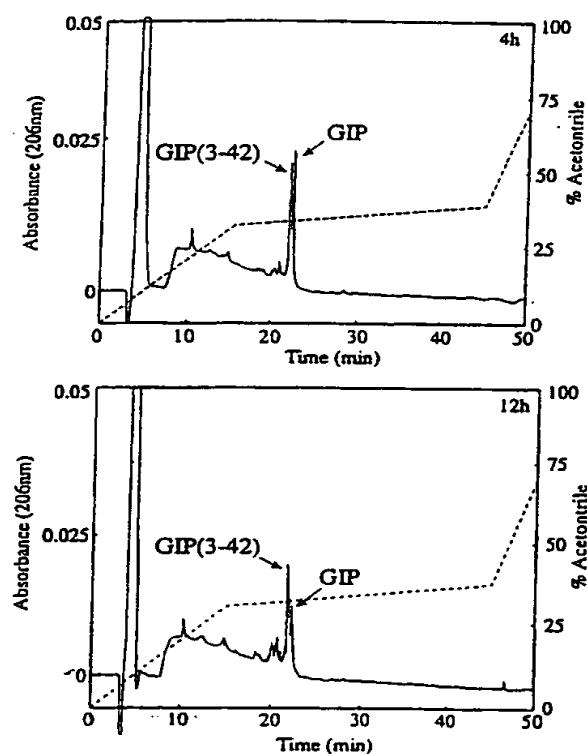
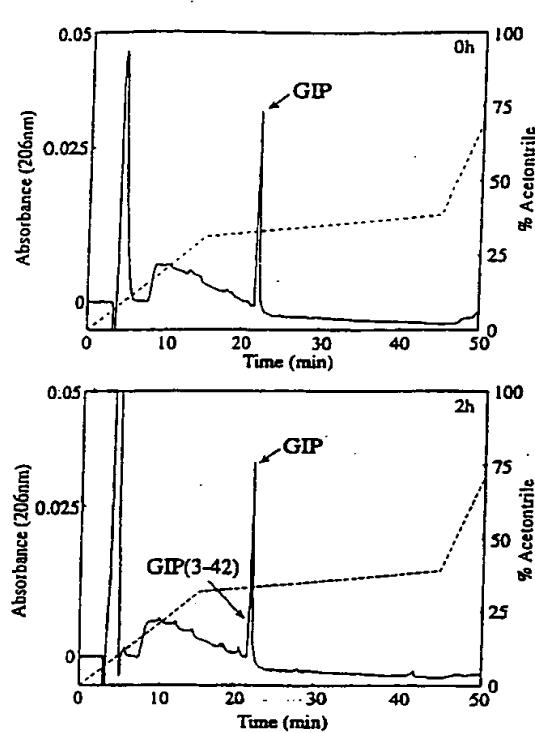
15

16 10. A method of N-terminally modifying GIP or
17 analogues thereof the method comprising the steps
18 of synthesising the peptide from the C terminal to
19 the penultimate N terminal amino acid, adding
20 tyrosine as a F-moc protected Tyr(tBu)-Wang resin,
21 deprotecting the N-terminus of the tyrosine and
22 reacting with modifying agent, allowing the
23 reaction to proceed to completion, cleaving the
24 modified tyrosine from the Wang resin and adding
25 the modified tyrosine to the peptide synthesis
26 reaction.

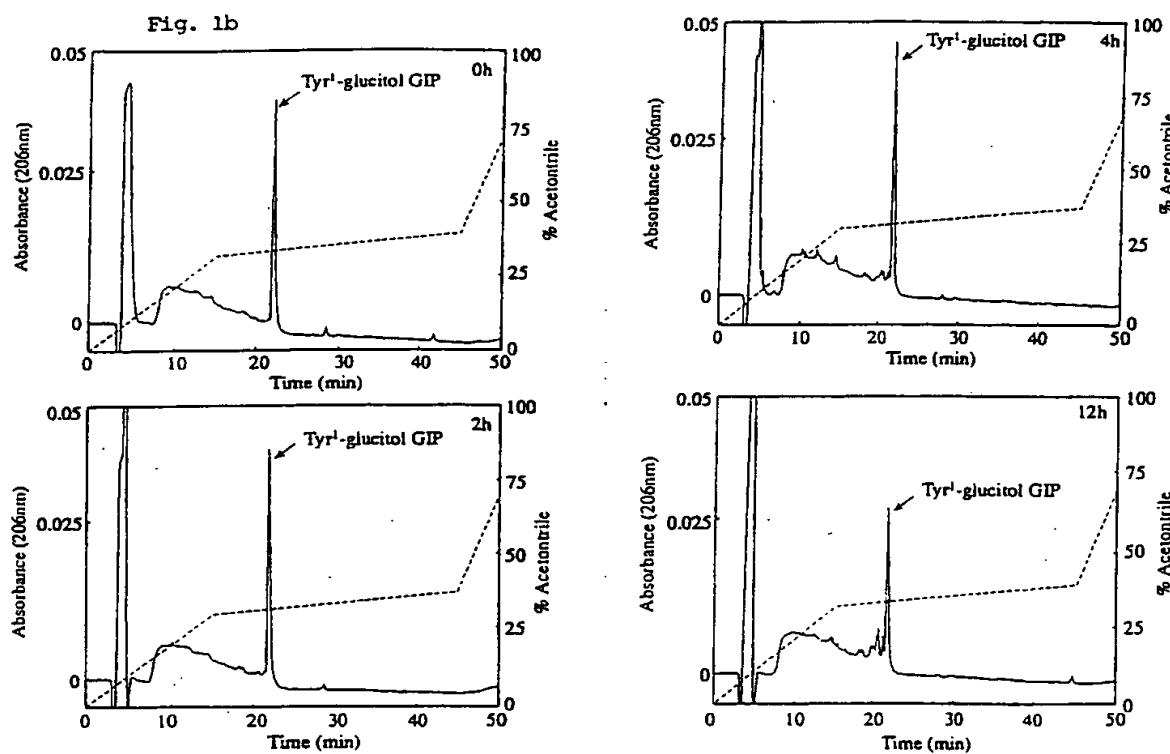
27

28 11. A method as claimed in claim 10 wherein the
29 modifying agent is chosen from the group
30 comprising glucose, acetic anhydride or
31 pyroglutamic acid.

Fig. 1a

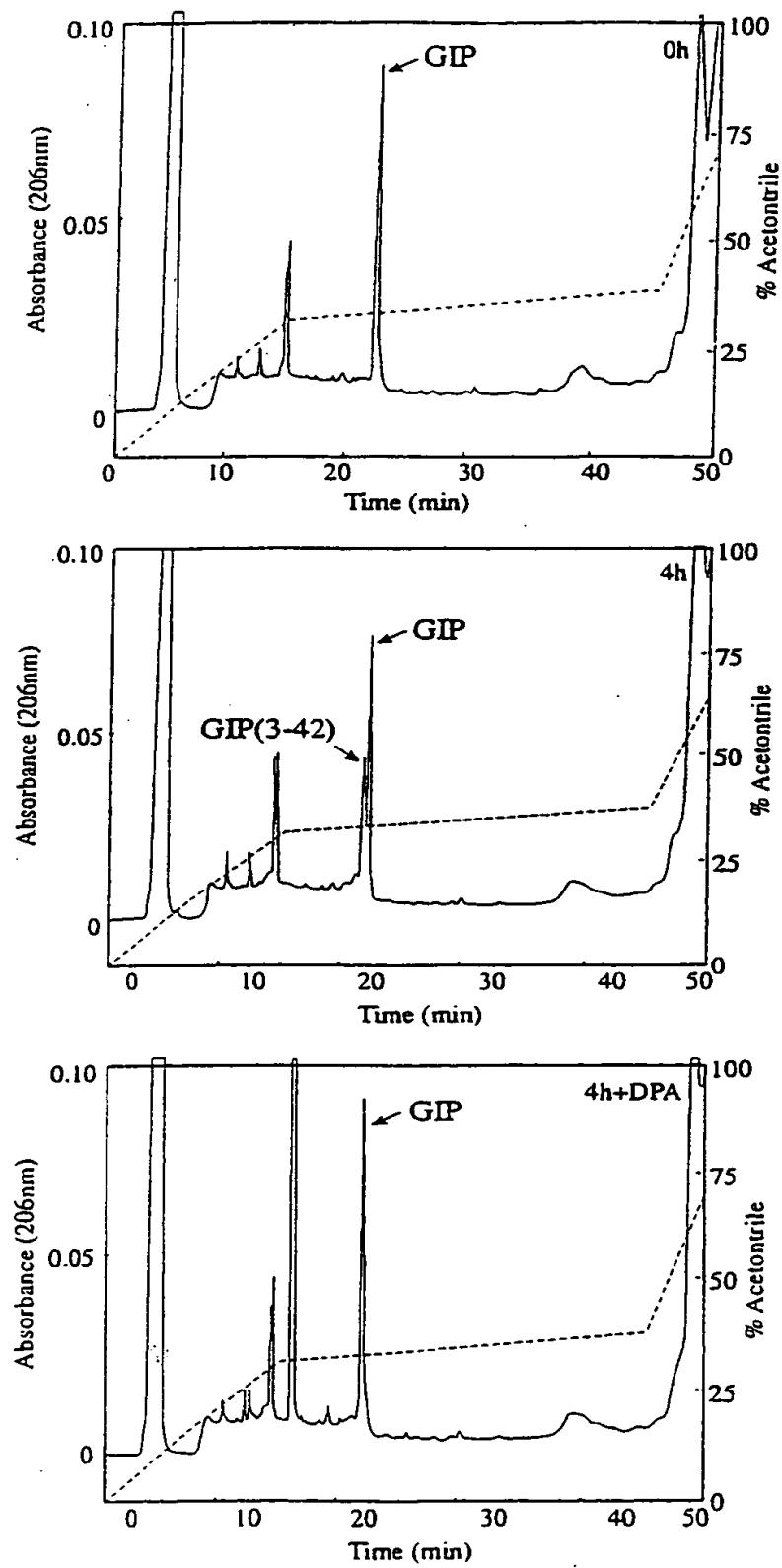


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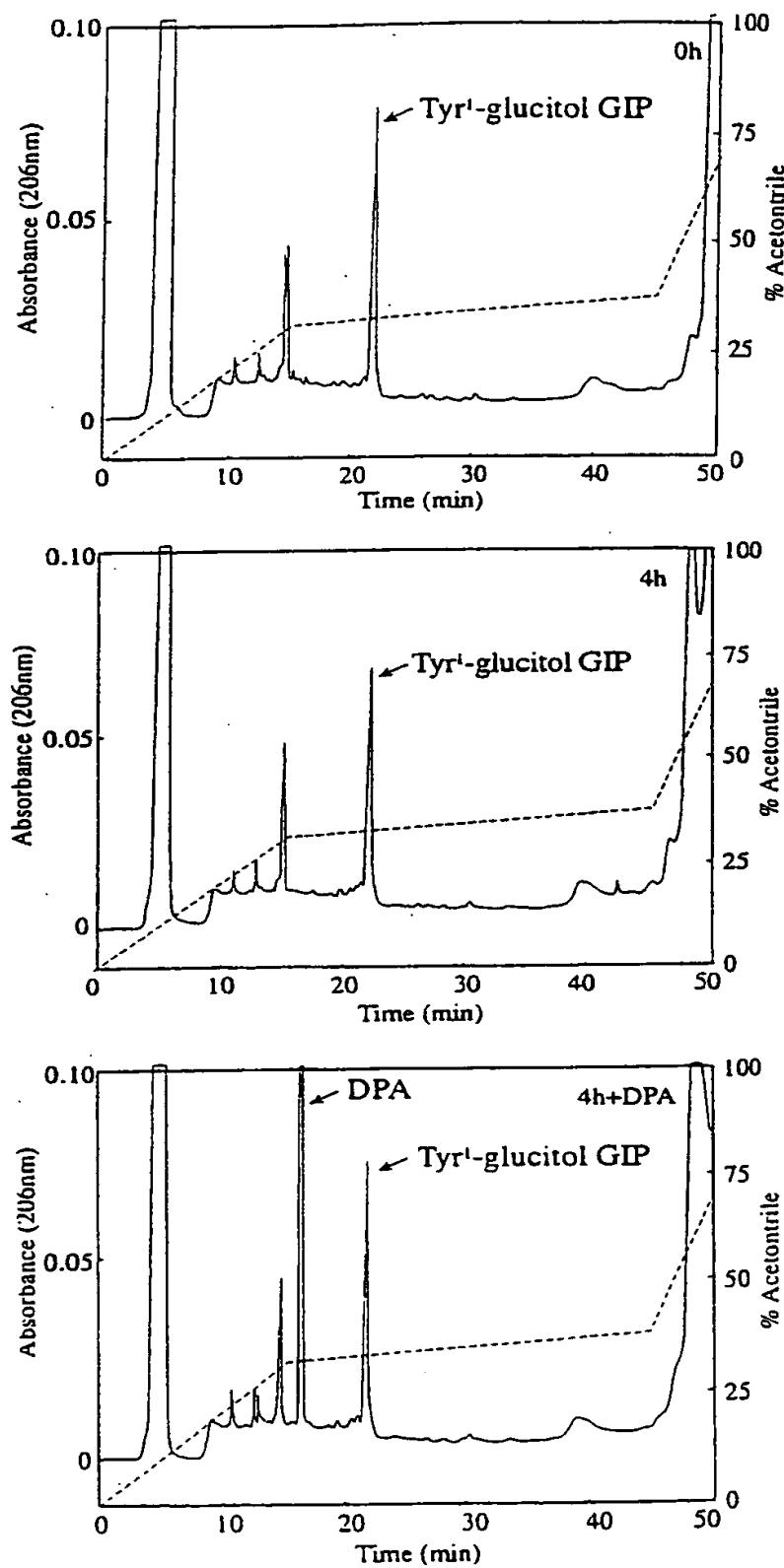
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Fig. 2a



4/32

Fig. 2b



5/32

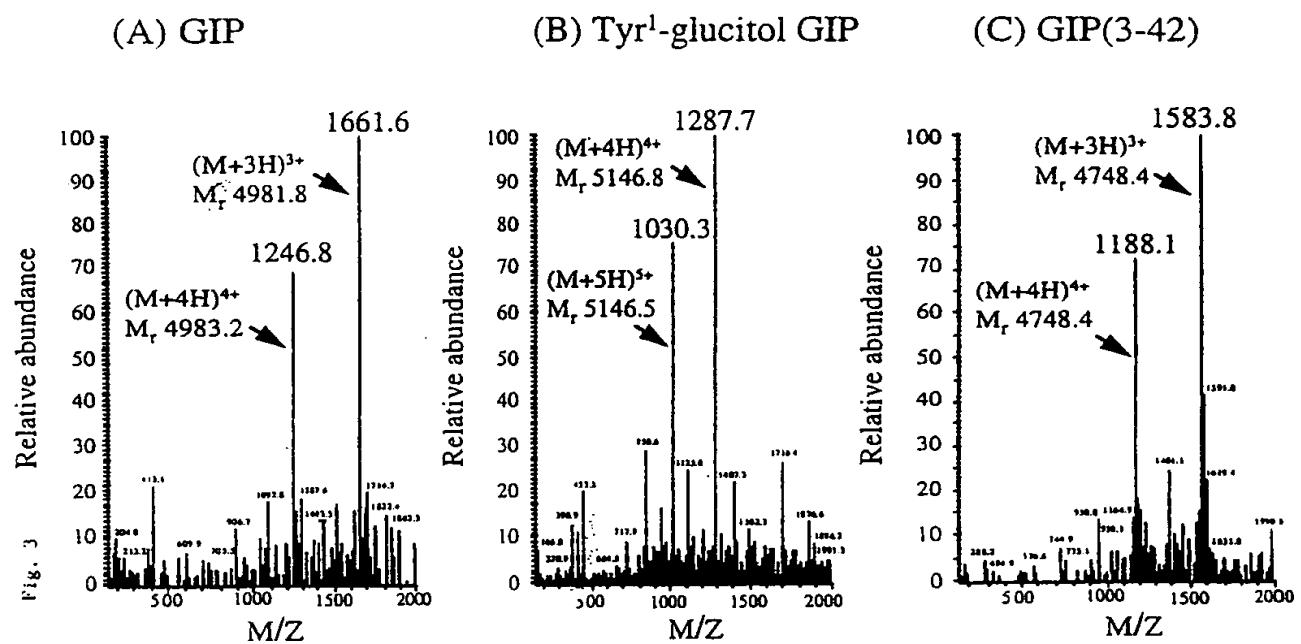
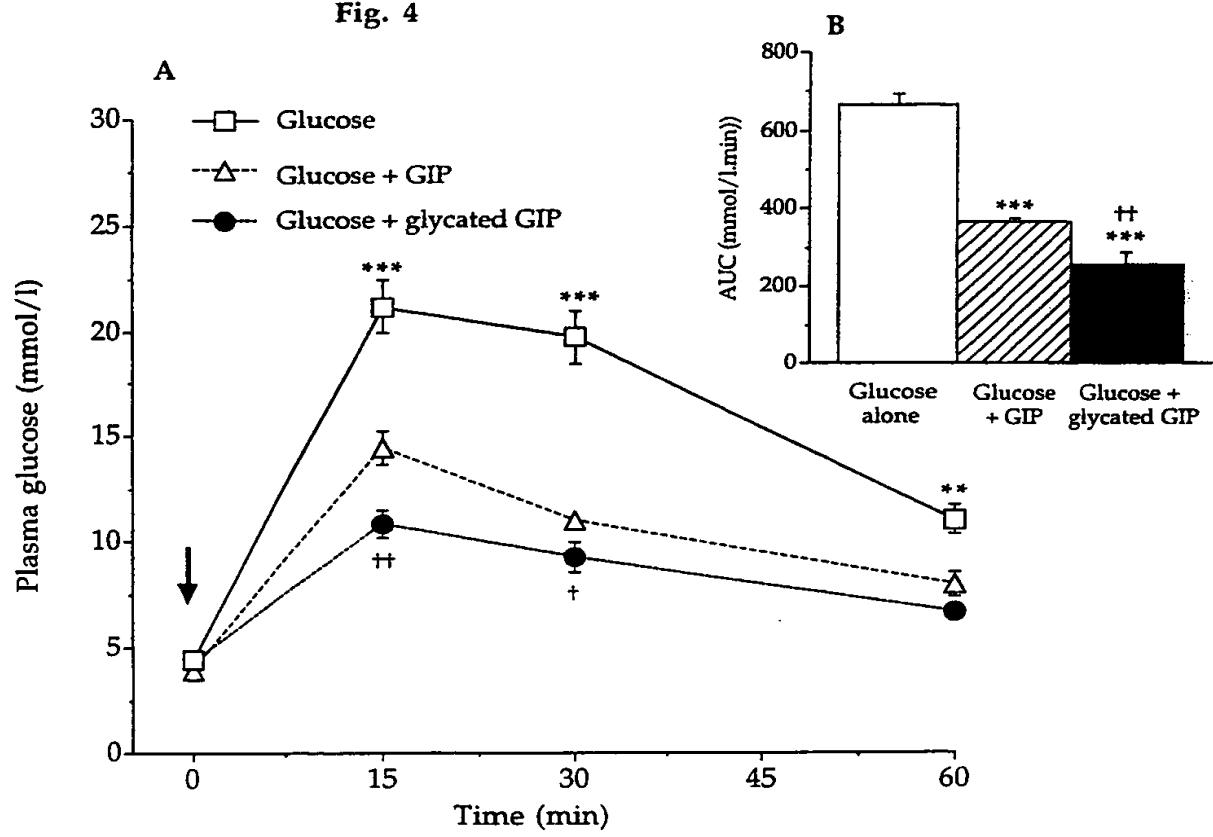
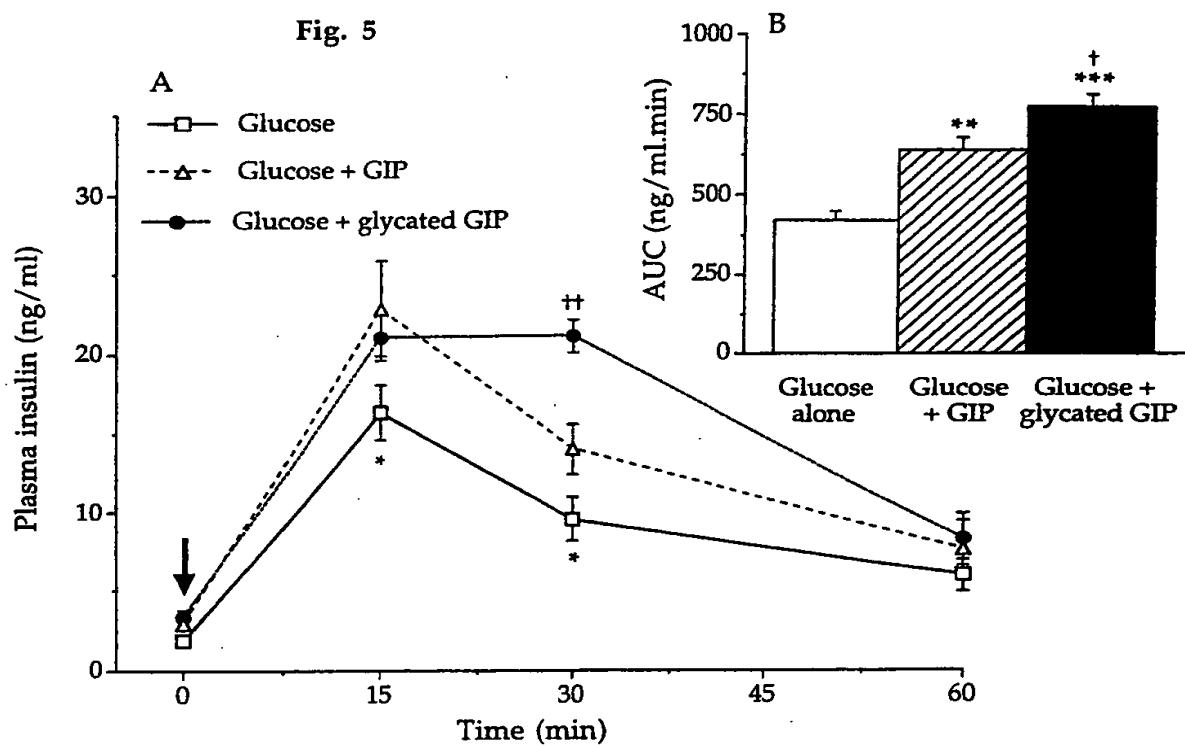


Fig. 4





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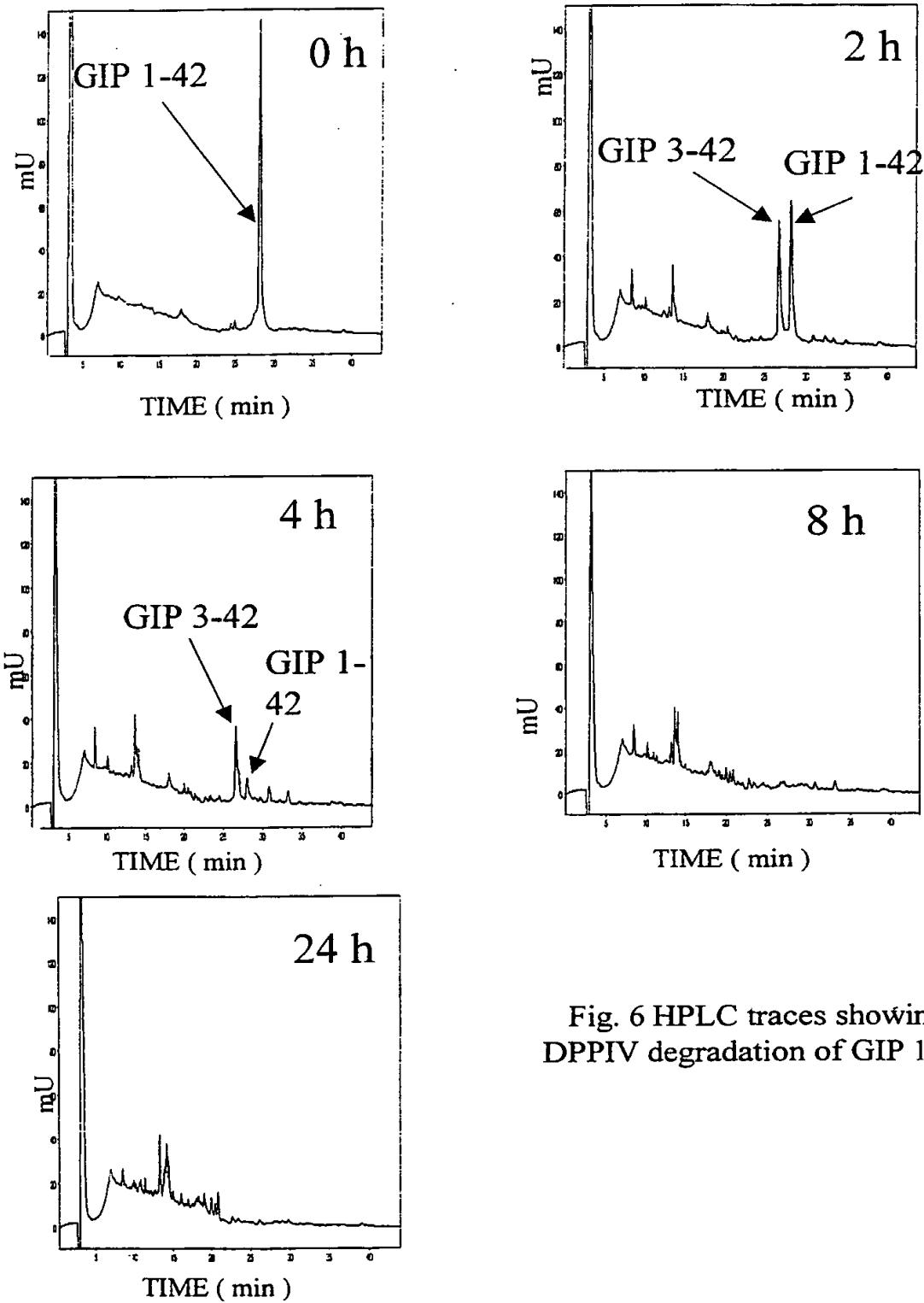


Fig. 6 HPLC traces showing DPPIV degradation of GIP 1-42

9 / 32

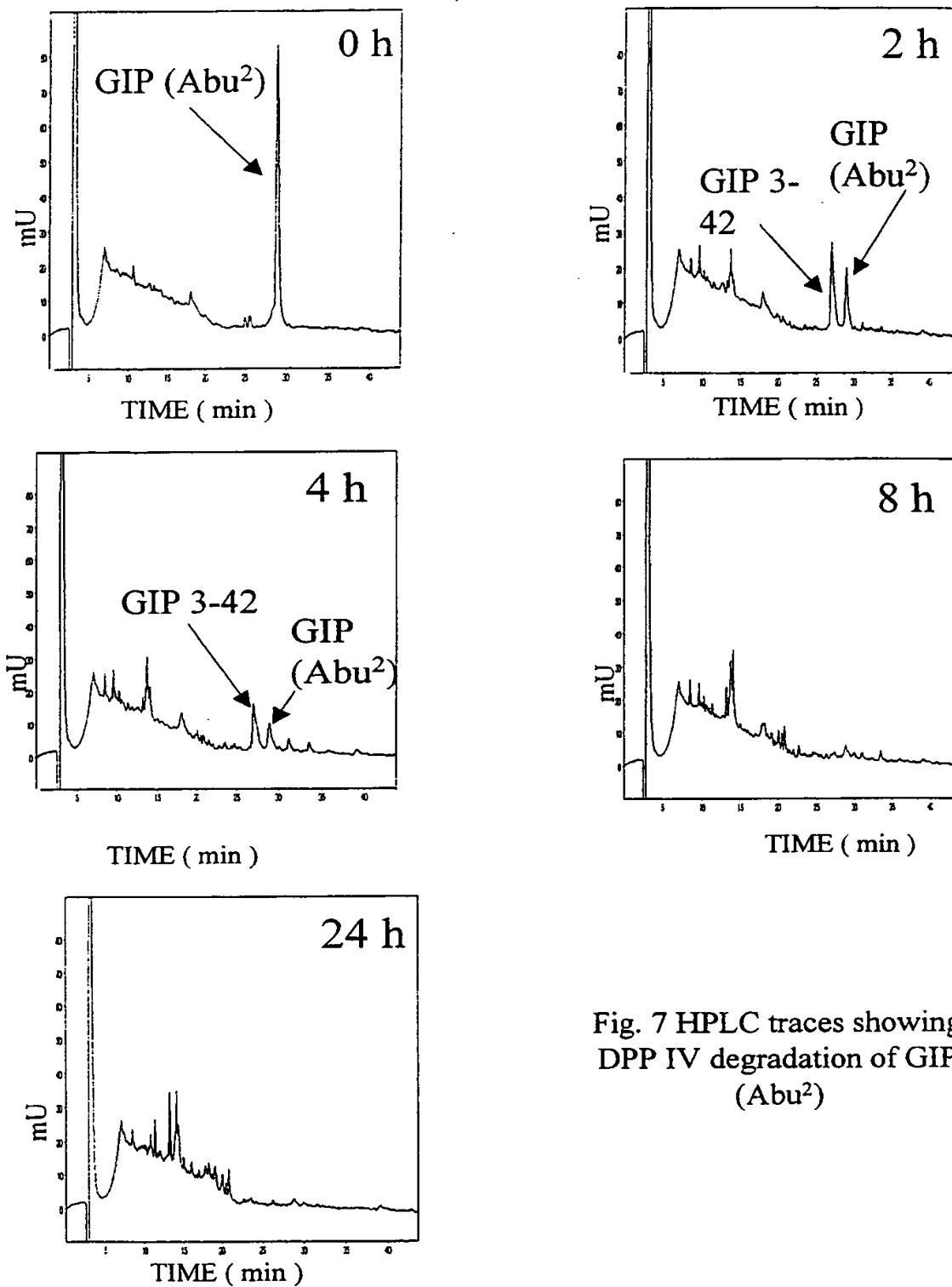


Fig. 7 HPLC traces showing DPP IV degradation of GIP (Abu^2)

10 / 32

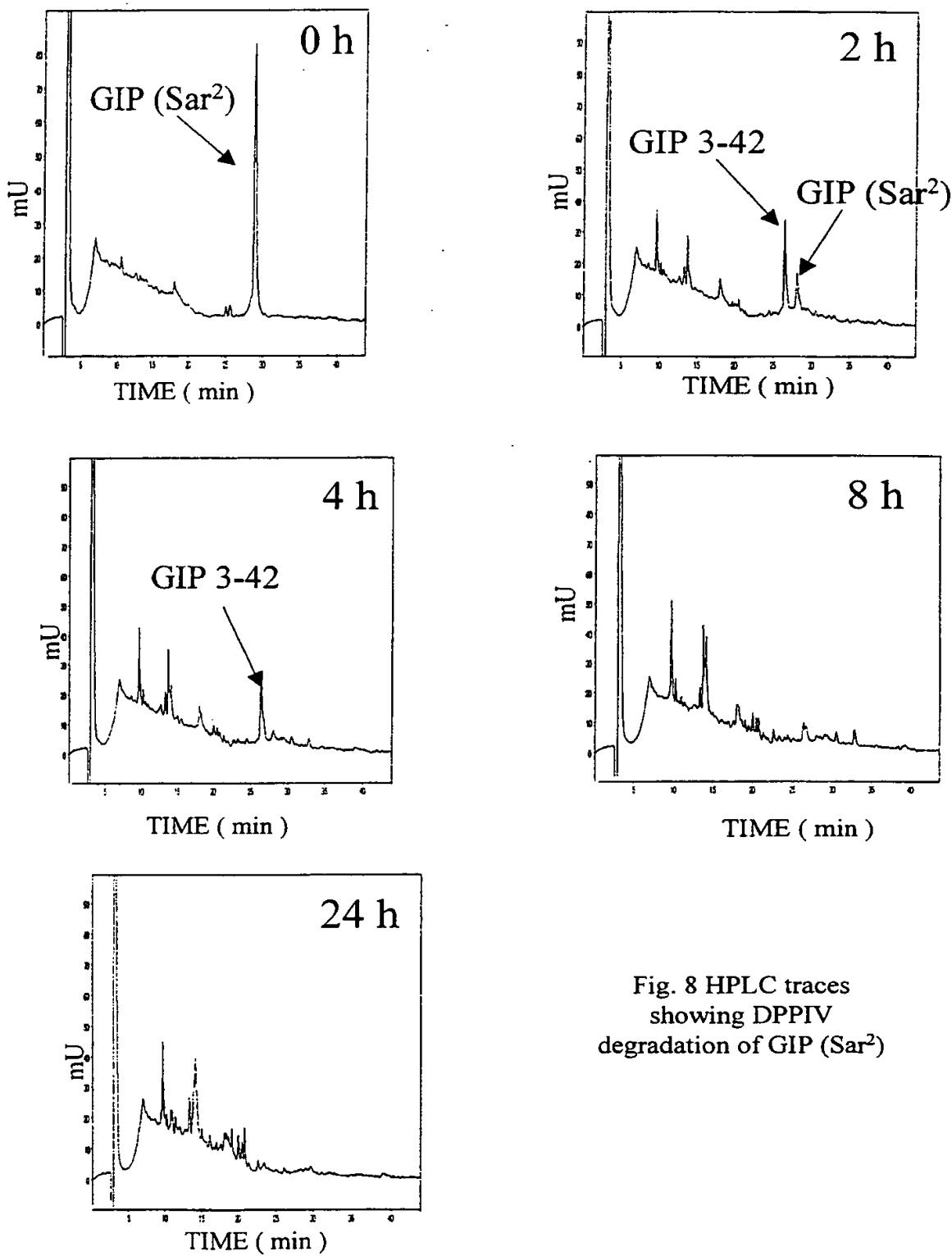


Fig. 8 HPLC traces
showing DPPIV
degradation of GIP (Sar^2)

11 / 32

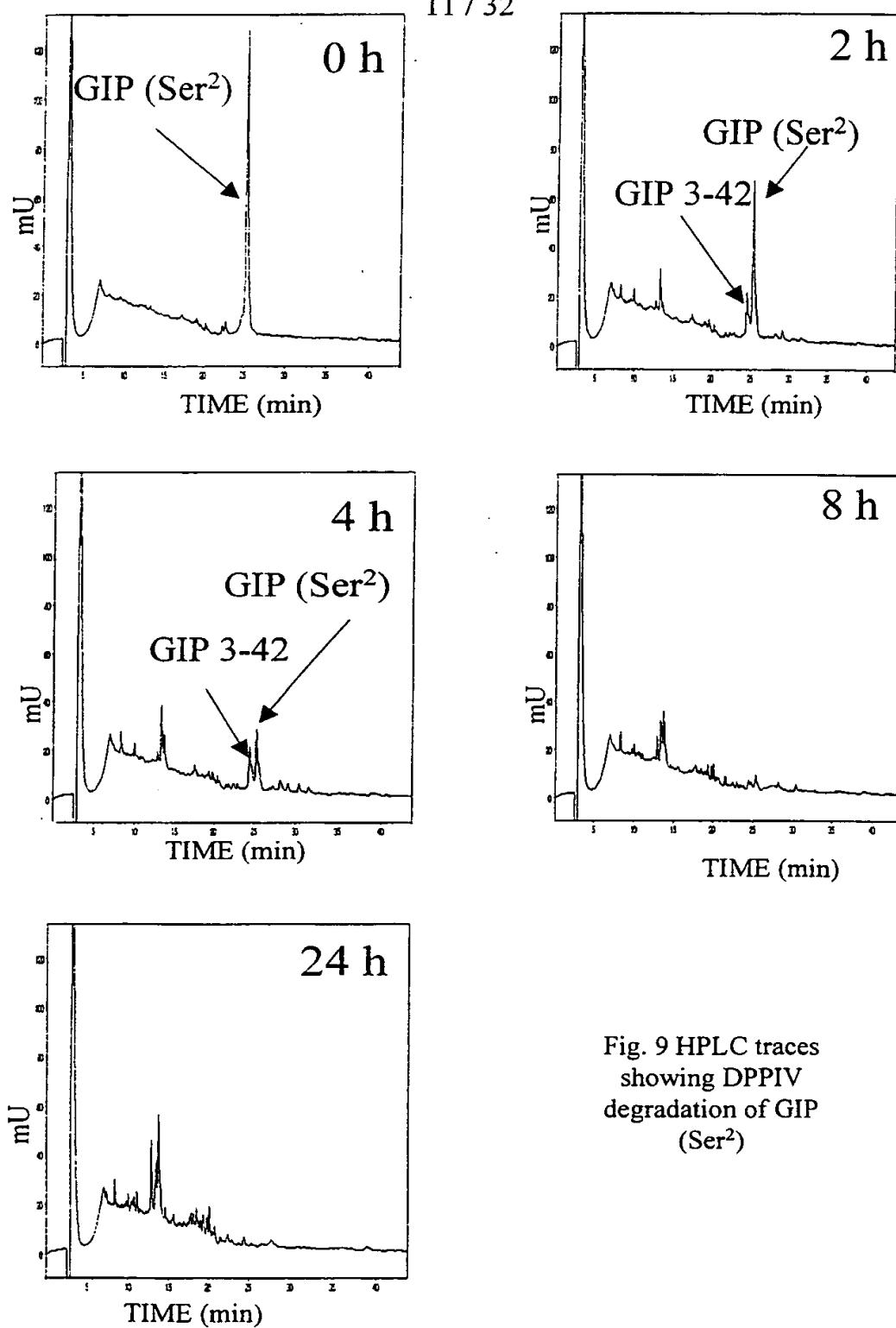


Fig. 9 HPLC traces
showing DPPIV
degradation of GIP
(Ser²)

12 / 32

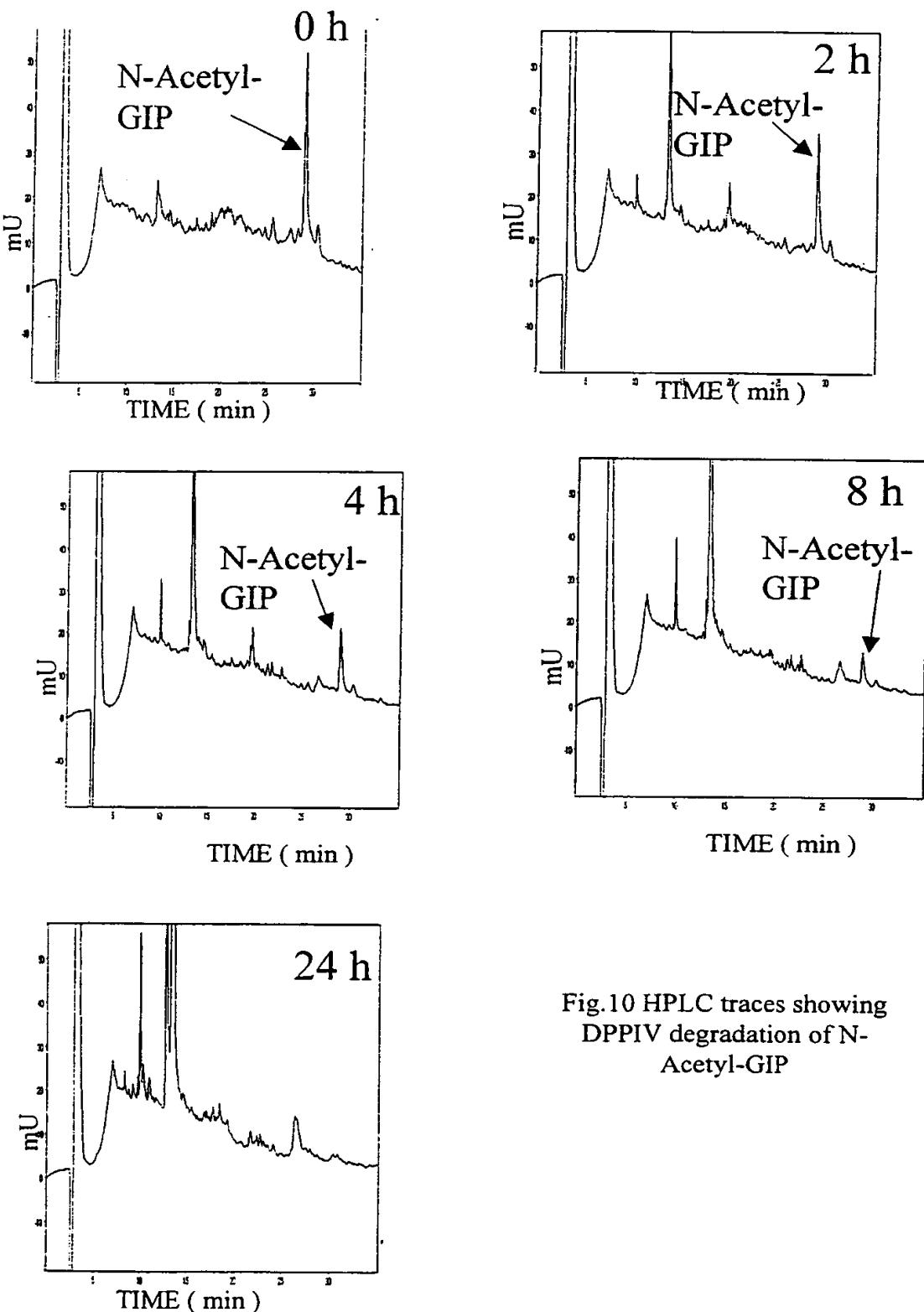


Fig.10 HPLC traces showing
DPPIV degradation of N-
Acetyl-GIP

13 / 32

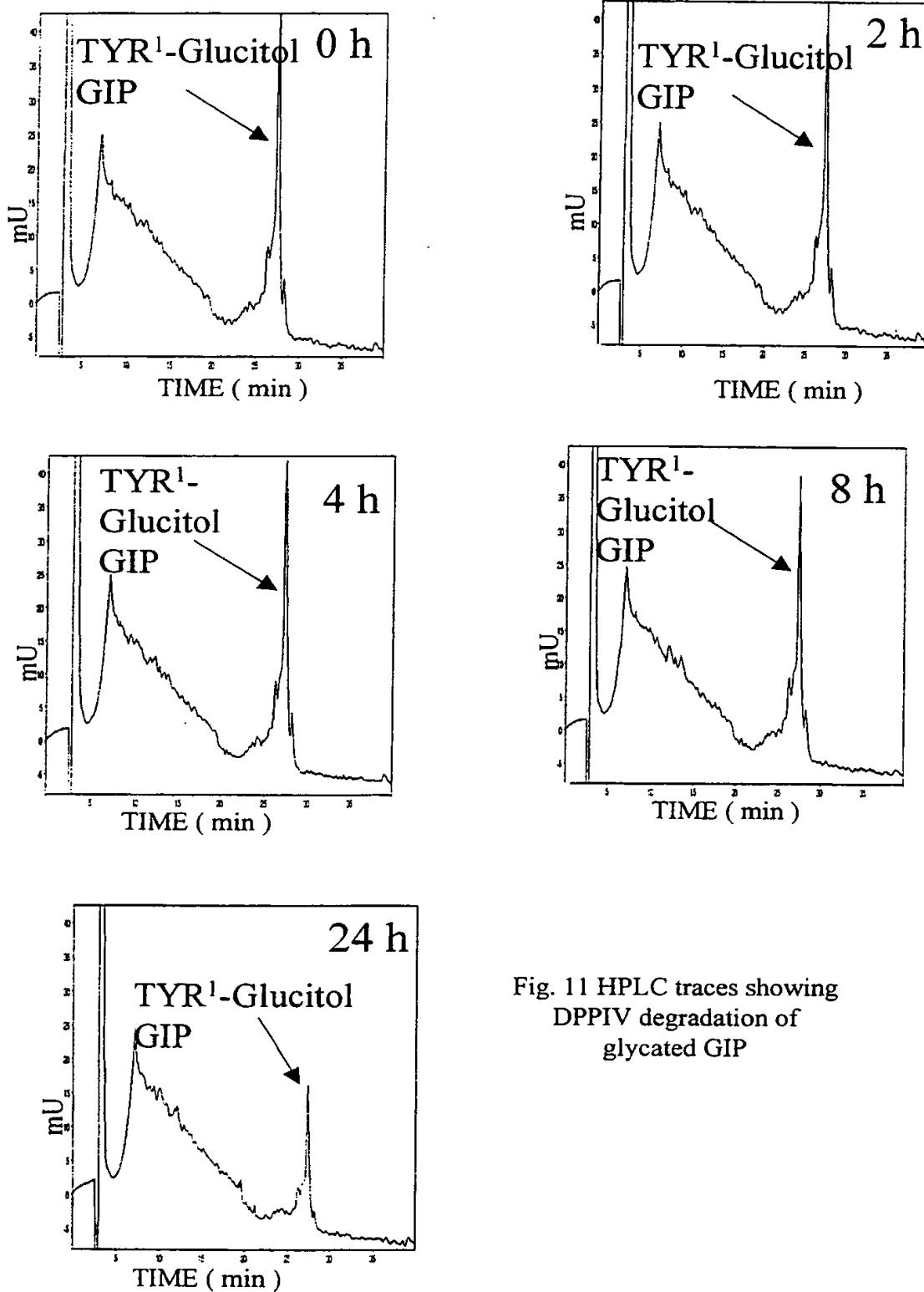


Fig. 11 HPLC traces showing
DPPIV degradation of
glycated GIP

14 / 32

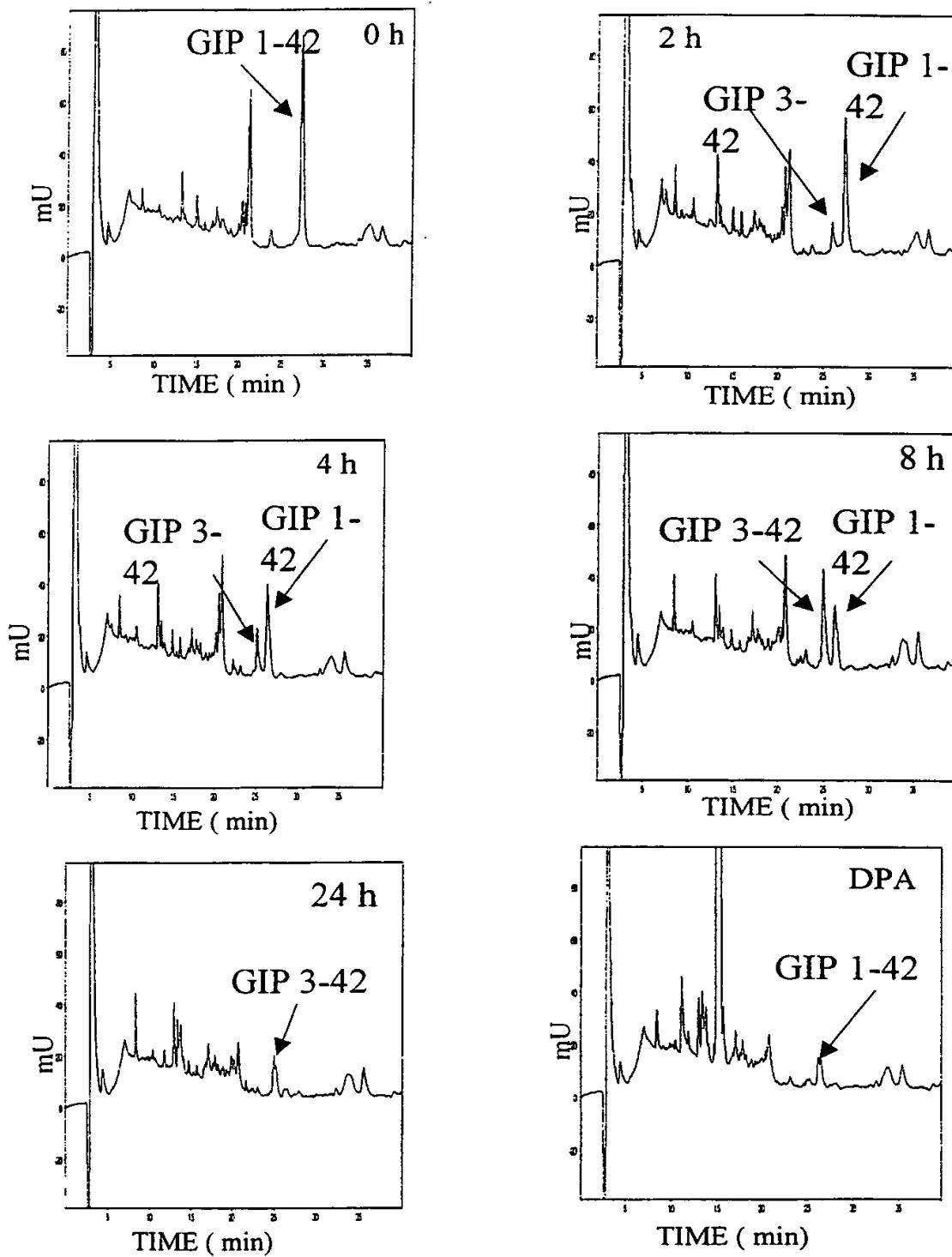


Fig.12. HPLC traces showing human plasma degradation of GIP

15 / 32

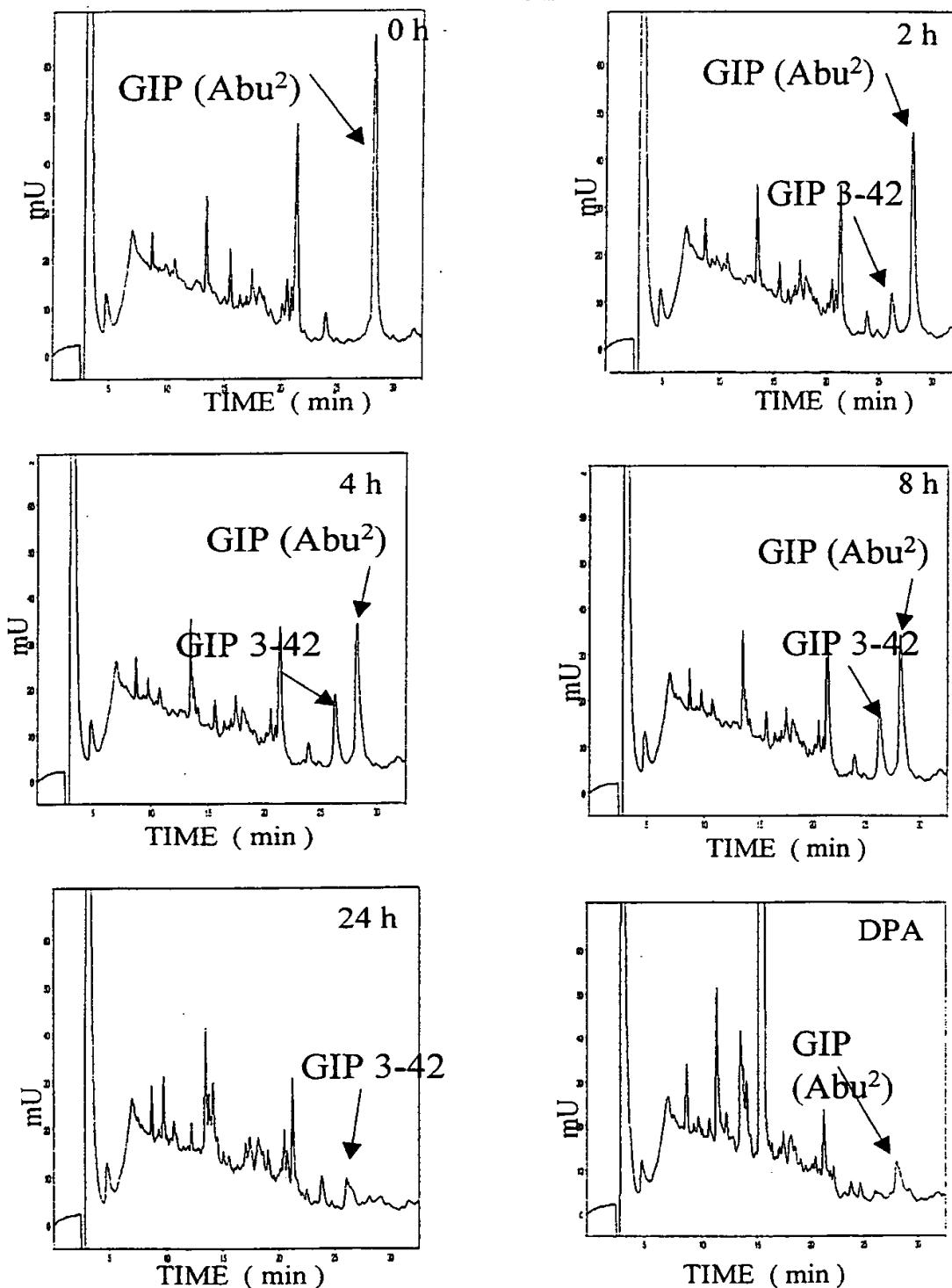
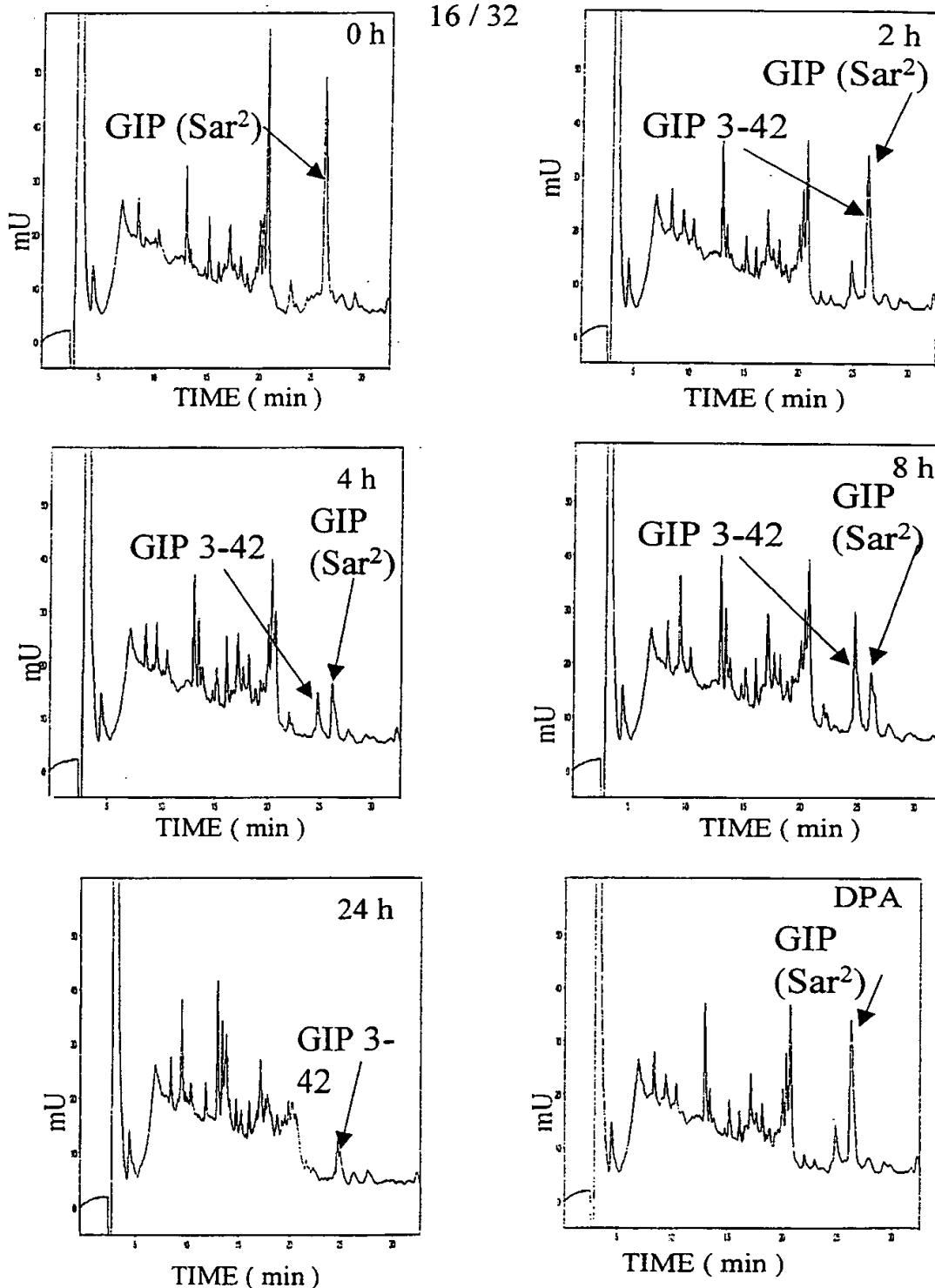


Fig. 13. HPLC traces showing human plasma degradation of GIP (Abu^2)

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Fig. 14. HPLC traces showing human plasma degradation of GIP (Sar²)

17 / 32

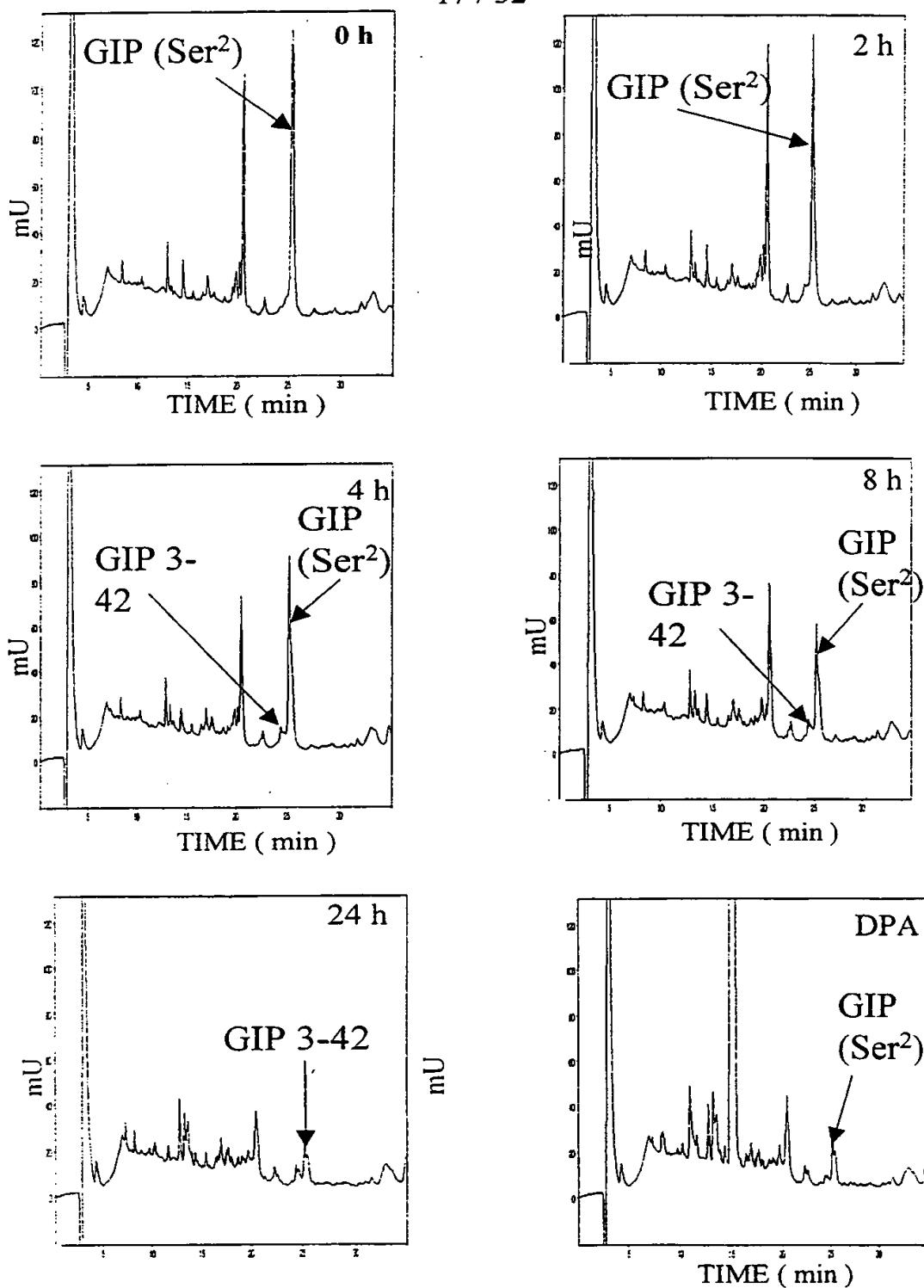


Fig. 15 HPLC traces showing human plasma degradation of GIP(Ser²)

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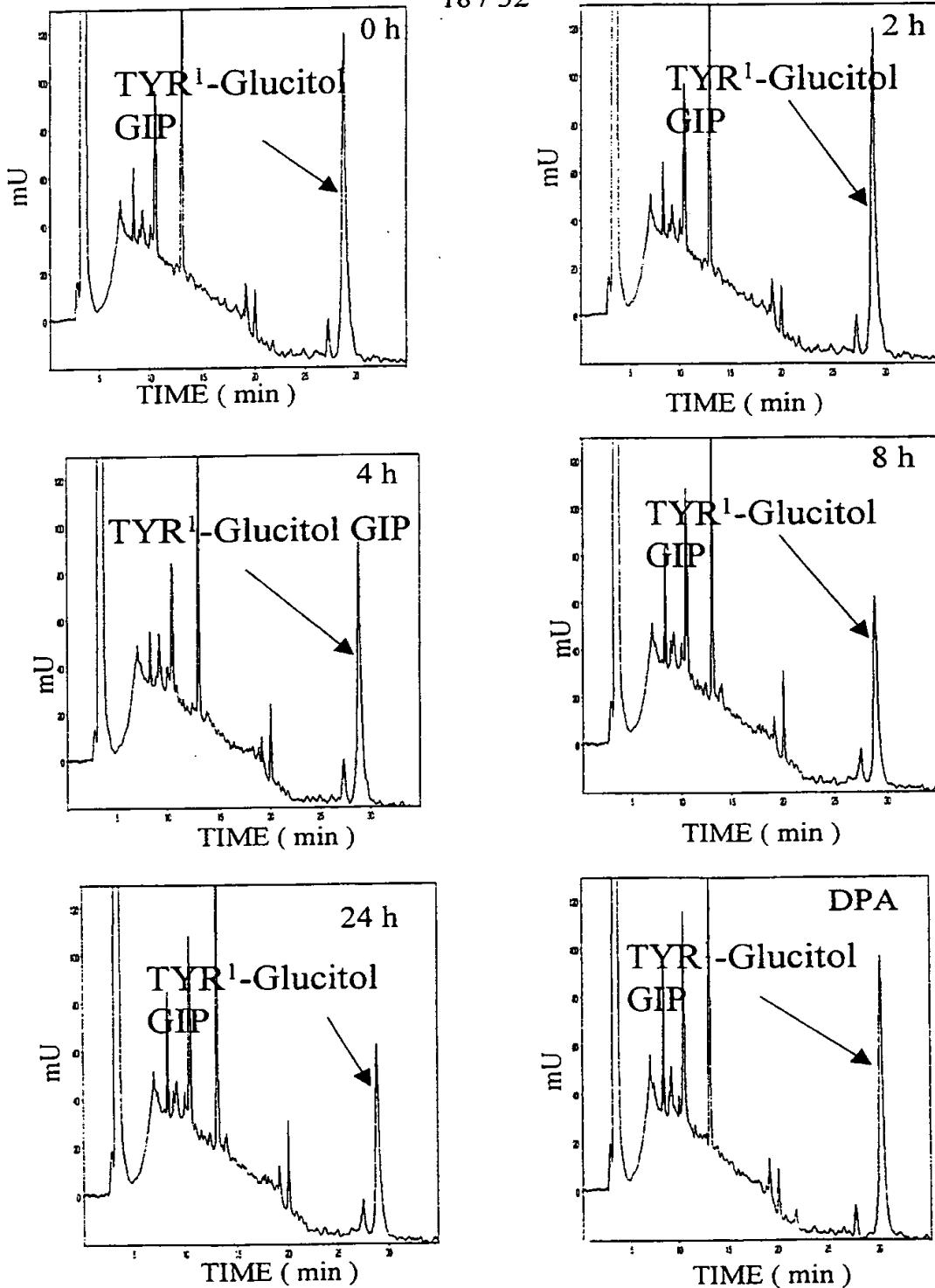
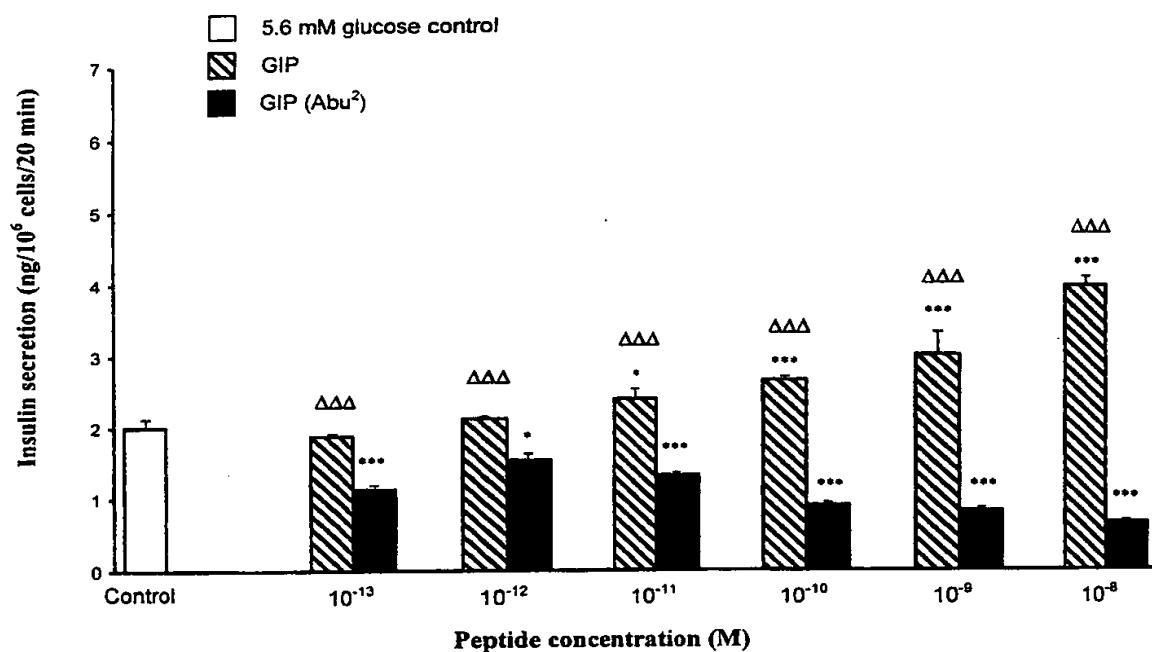


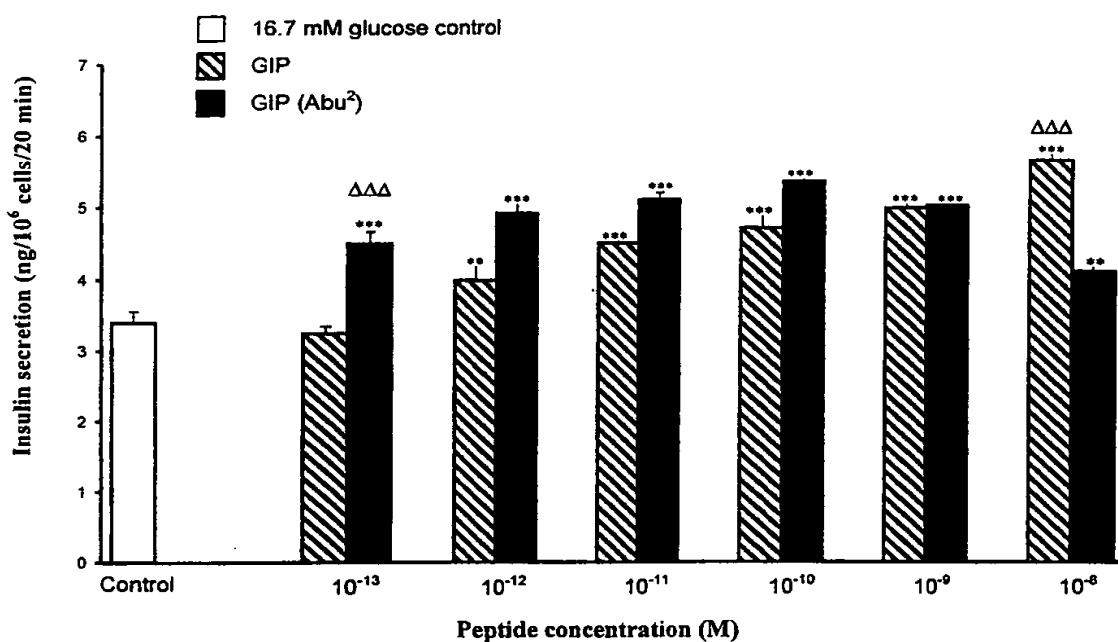
Fig. 16. HPLC traces showing human plasma degradation of glycated GIP

Fig.17. Graph showing the effects of various concentrations of GIP and GIP (Abu^2) on insulin release from BRIN-BD11 cells incubated at 5.6 mM glucose



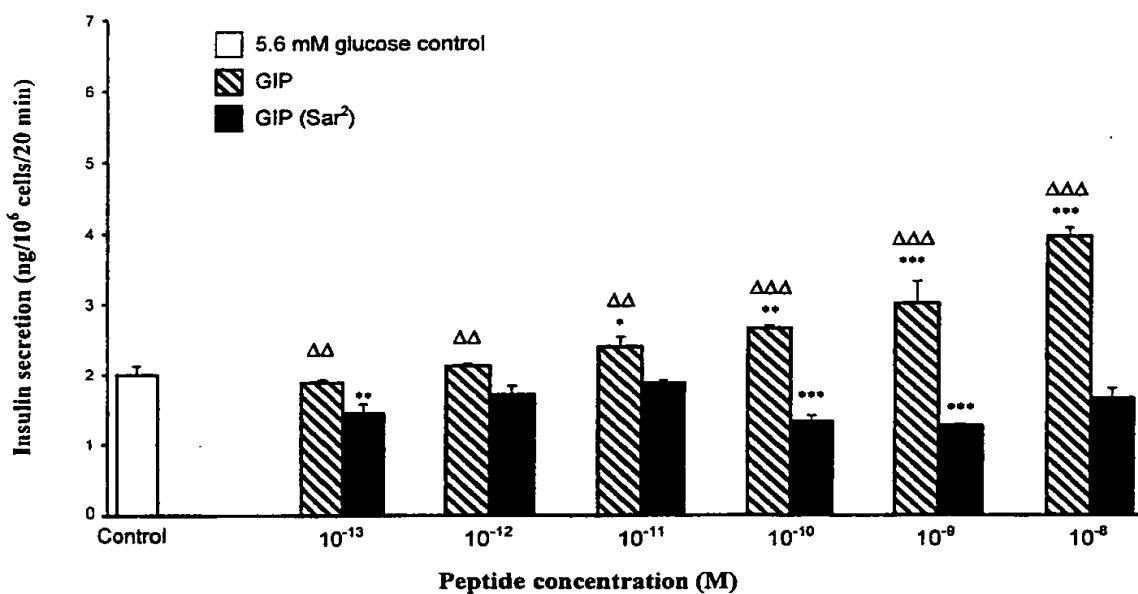
Values are means \pm S.E.M. for 12 separate observations. *P<0.05, **P<0.01, ***P<0.001 compared to control (5.6mM glucose alone). ^P<0.05, ^AP<0.01, ^AAAP<0.001 compared to GIP (Abu^2) at the same concentration.

Fig.18. Graph showing the effects of various concentrations of GIP and GIP (Abu²) on insulin release from BRIN-BD11 cells incubated at 16.7 mM glucose.



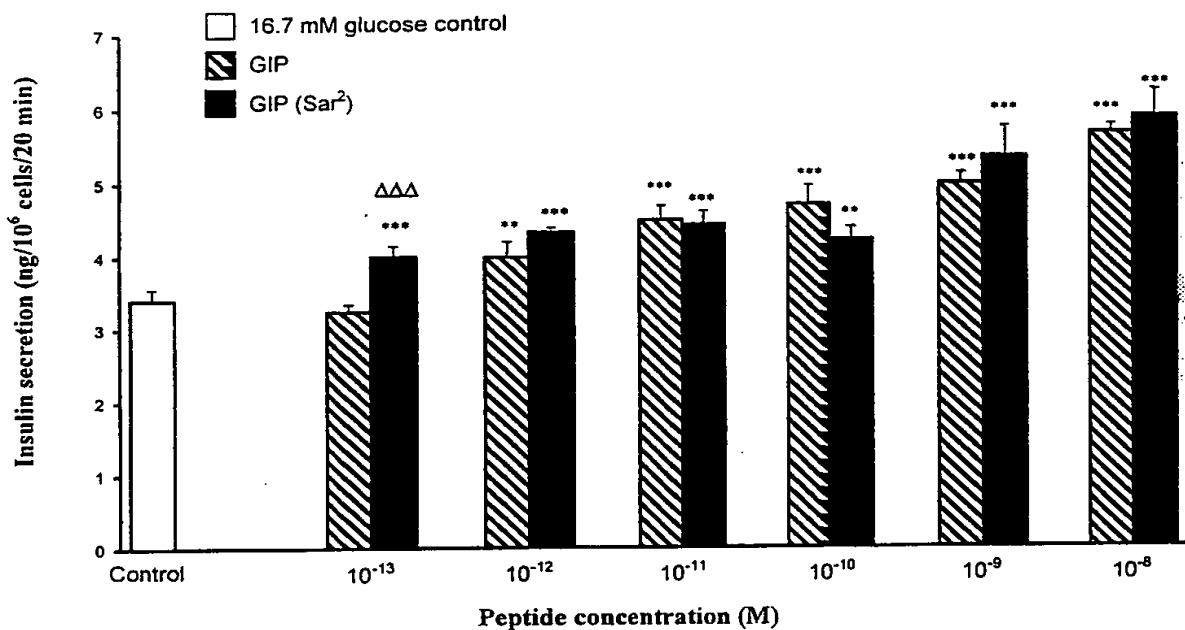
Values are means \pm S.E.M. for 12 separate observations. *P< 0.05, **P< 0.01, ***P<0.001 compared to control (16.7 mM glucose alone). ΔP<0.05, ΔΔP<0.01, ΔΔΔP<0.001 compared to GIP (Abu²) at the same concentration.

Fig.19. Graph showing the effects of various concentrations of GIP and GIP (Sar^2) on insulin release from BRIN-BD11 cells incubated at 5.6 mM glucose



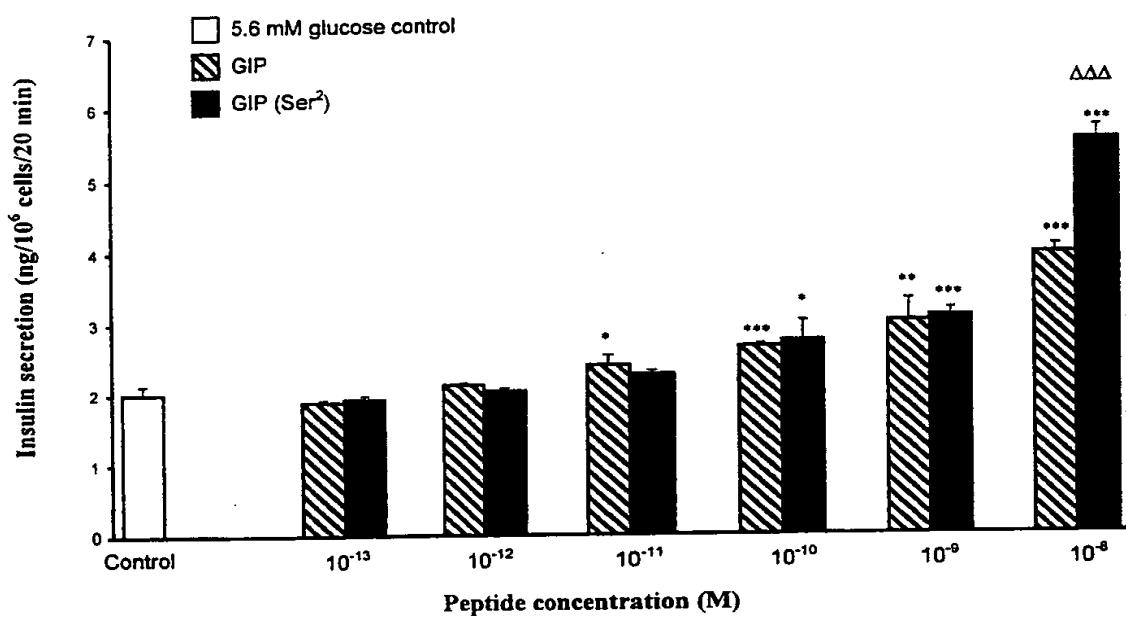
Values are means \pm S.E.M. for 12 separate observations. *P<0.05, **P<0.01, ***P<0.001 compared to control (5.6mM glucose alone). ΔP<0.05, ΔΔP<0.01, ΔΔΔP<0.001 compared to GIP (Sar^2) at the same concentration.

Fig. 20. Graph showing the effects of various concentrations of GIP and GIP (Sar^2) on insulin release from BRIN-BD11 cells incubated at 16.7 mM glucose



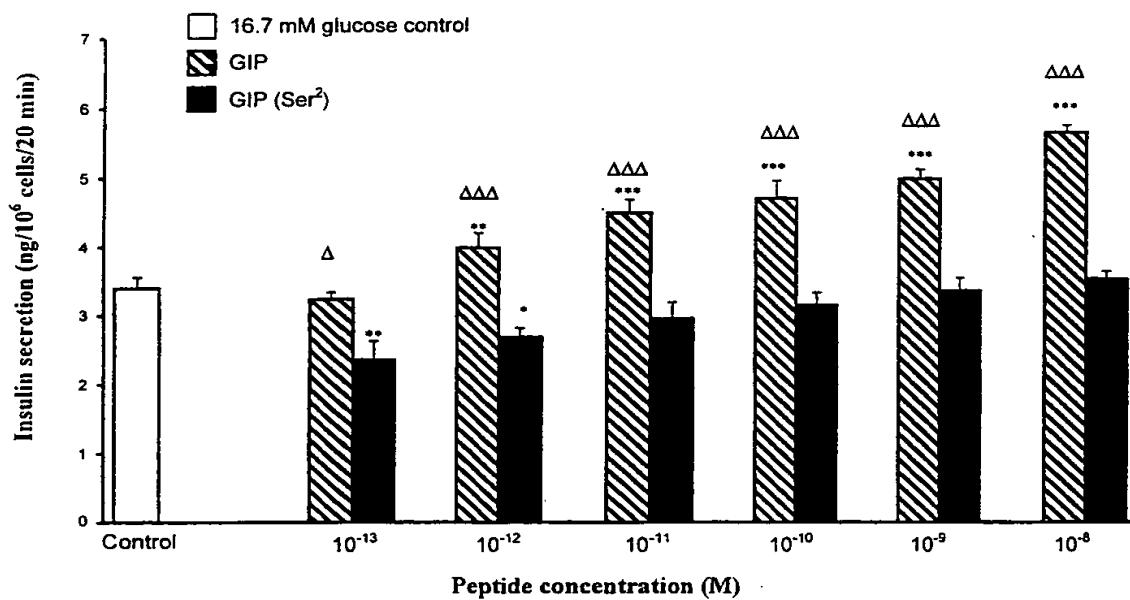
Values are means \pm S.E.M. for 12 separate observations. $^{\wedge}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ compared to control (16.7 mM glucose alone). $^{\wedge}P<0.05$, $^{\wedge\wedge}P<0.01$, $^{\wedge\wedge\wedge}P<0.001$ compared to GIP (Sar^2) at the same concentration.

Fig.21. Graph showing the effects of various concentrations of GIP and GIP (Ser^2) on insulin release from BRIN-BD11 cells incubated at 5.6 mM glucose



Values are means \pm S.E.M. for 12 separate observations. *P<0.05, **P<0.01, ***P<0.001 compared to control (5.6mM glucose alone). *P<0.05, **P<0.01, ***P<0.001 compared to GIP (Ser^2) at the same concentration.

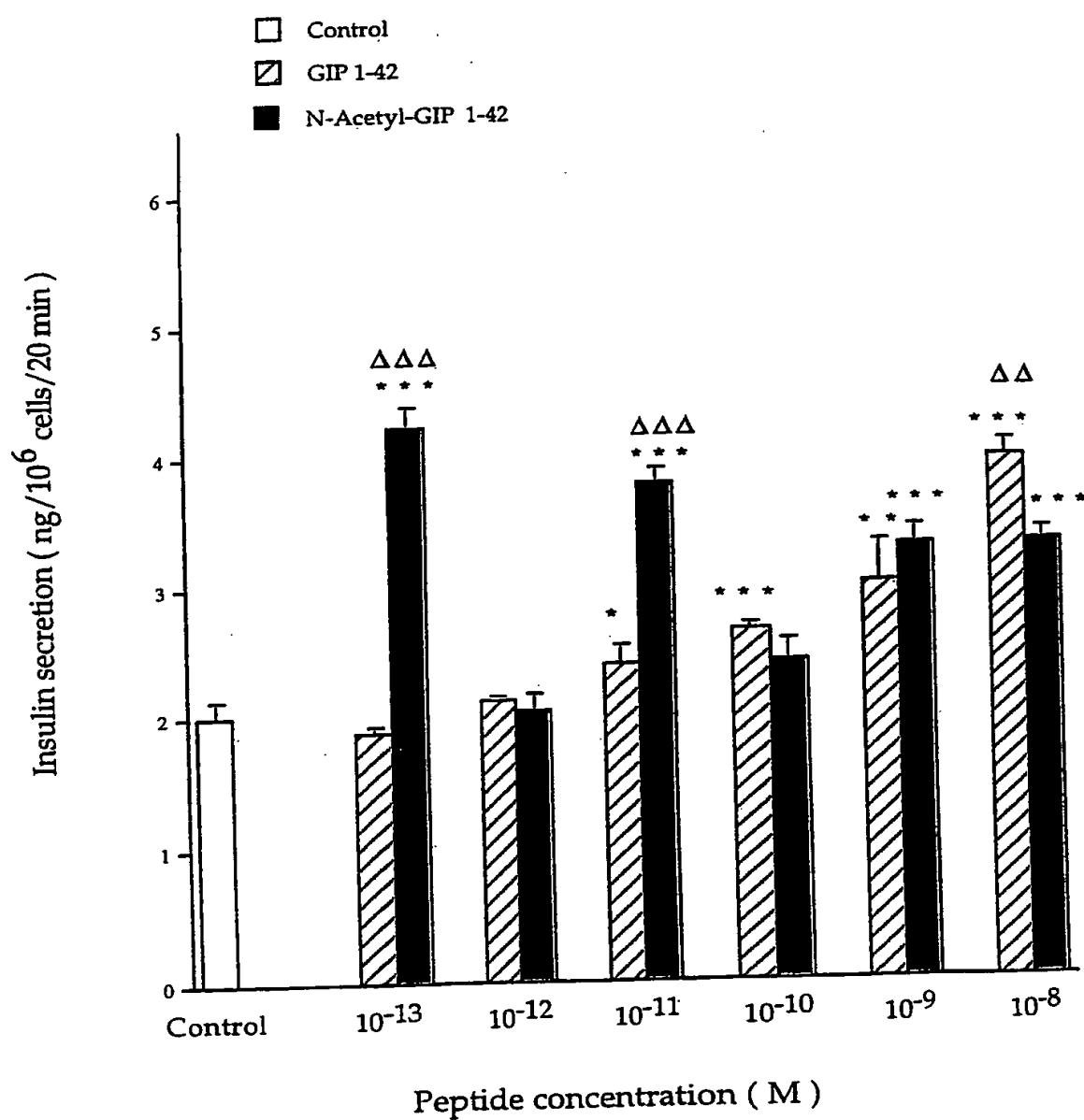
Fig. 22. Graph showing the effects of various concentrations of GIP and GIP (Ser²) on insulin release from BRIN-BD11 cells incubated at 16.7 mM glucose



Values are means \pm S.E.M. for 12 separate observations. *P<0.05, **P<0.01, ***P<0.001 compared to control (16.7 mM glucose alone). Δ P<0.05, $\Delta\Delta$ P<0.01, $\Delta\Delta\Delta$ P<0.001 compared to GIP (Ser²) at the same concentration.

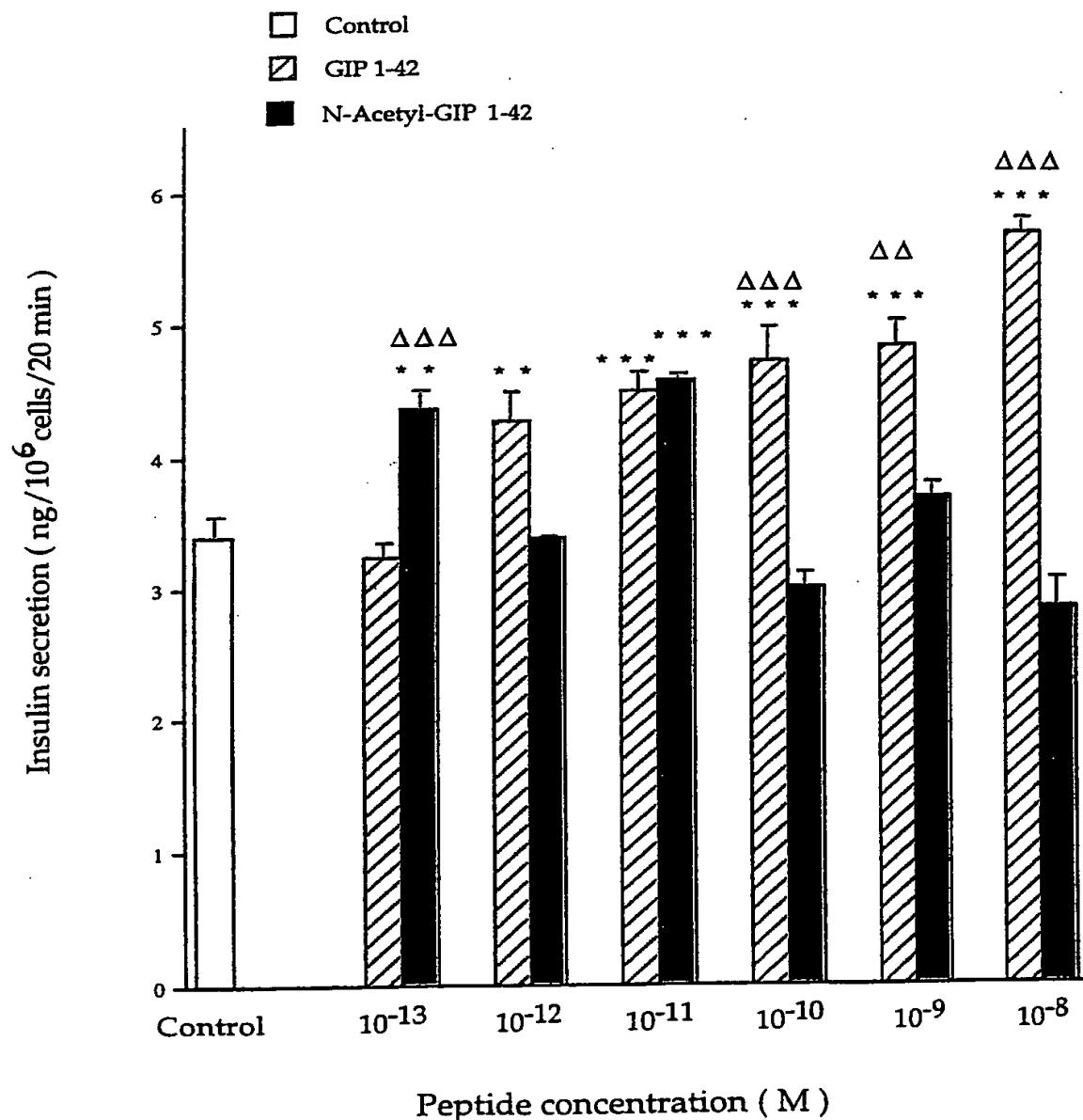
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Fig. 23 Graph showing the effects of various concentrations of GIP 1-42 and N-Acetyl-GIP 1-42 on insulin release from BRIN-BD11 cells incubated at 5.6 mM glucose



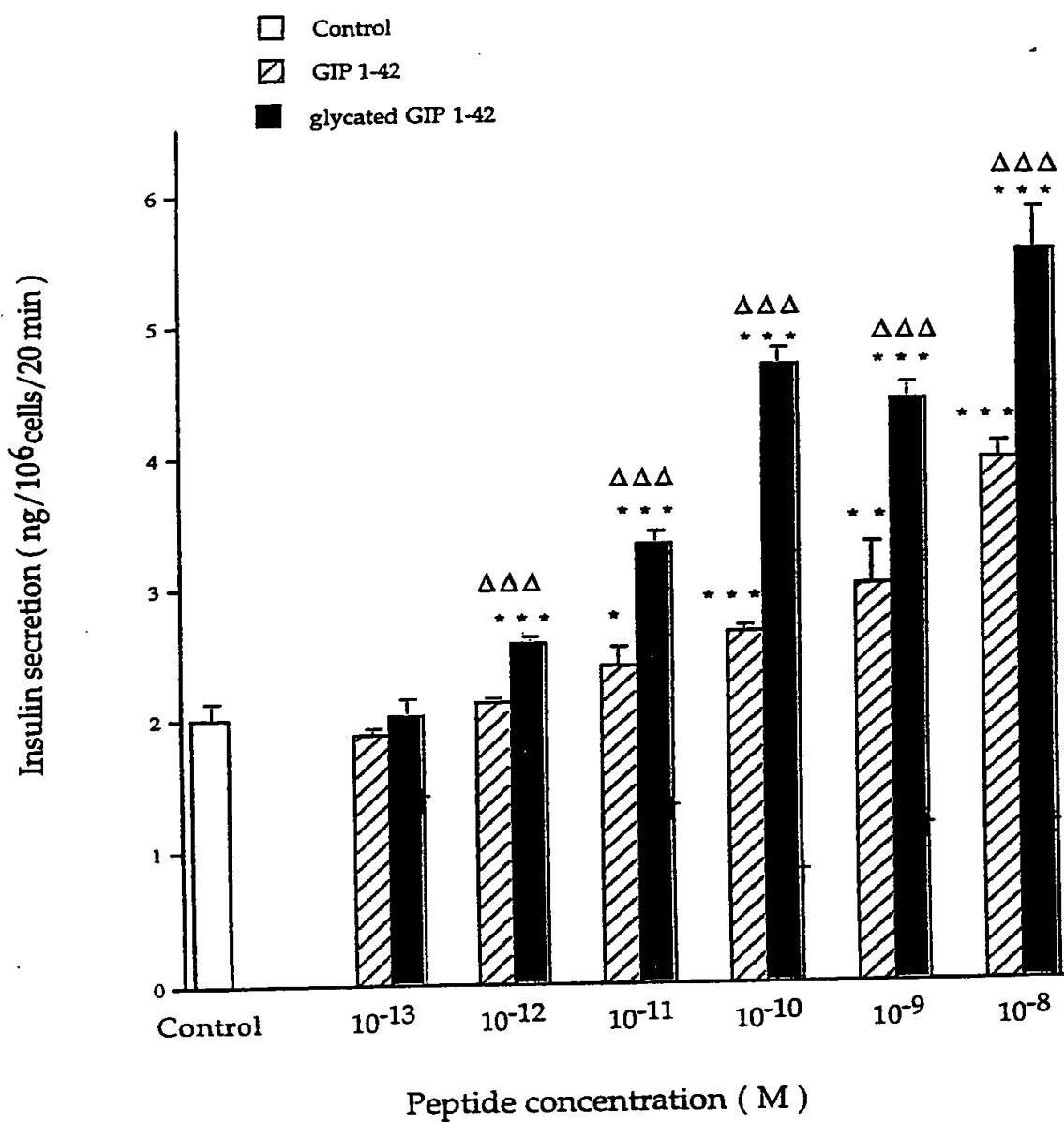
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Fig. 24 Graph showing the effects of various concentrations of GIP 1-42 and N-Acetyl-GIP 1-42 on insulin release from BRIN-BD11 cells incubated at 16.7 mM glucose



27/32

Fig. 25 Graph showing the effects of various concentrations of GIP 1-42 and glycated GIP 1-42 on insulin release from BRIN-BD11 cells incubated at 5.6 mM glucose



28/32

Fig. 26 Graph showing the effects of various concentrations of GIP 1-42 and glycated GIP 1-42 on insulin release from BRIN-BD11 cells incubated at 16.7 mM glucose

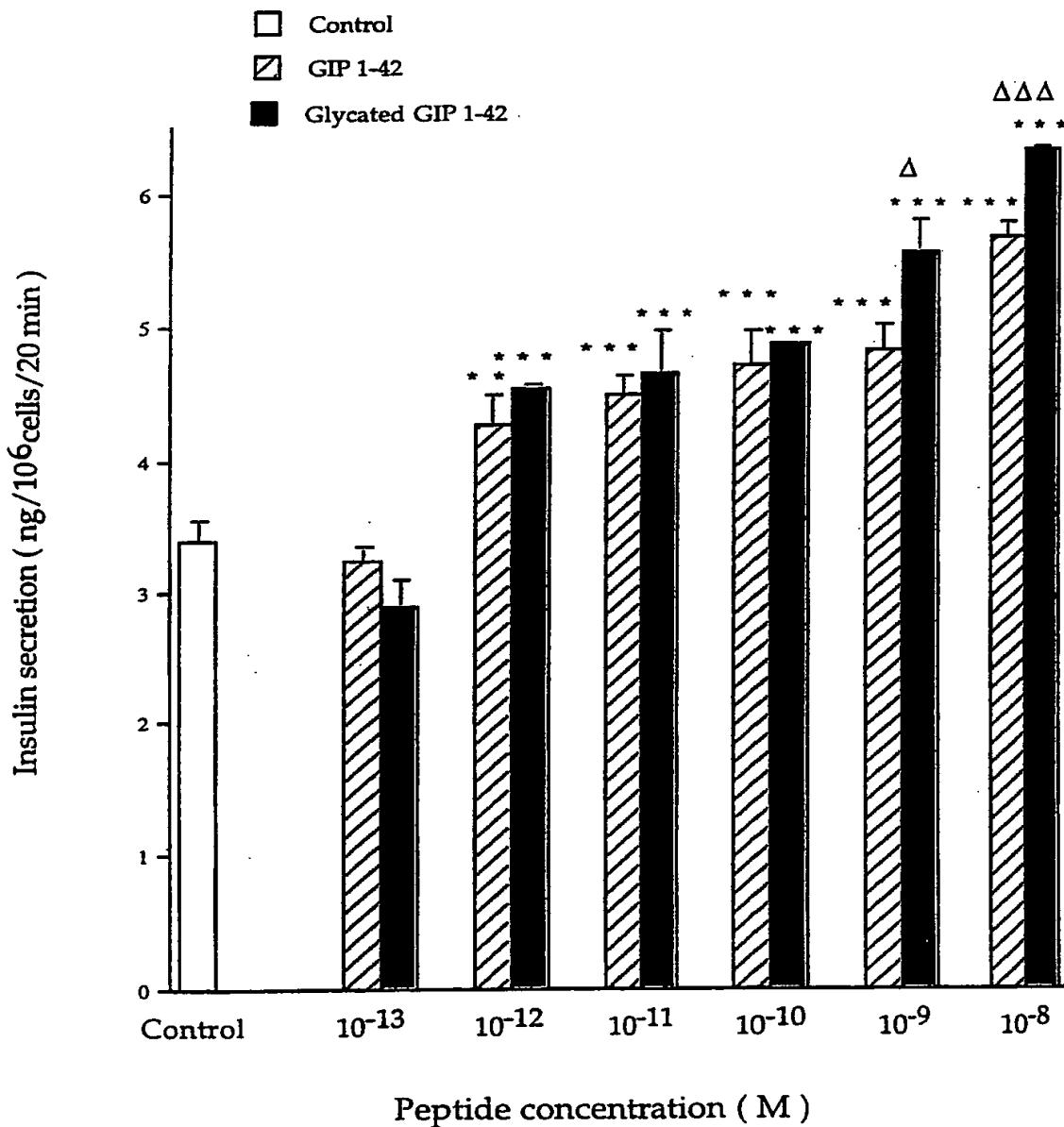
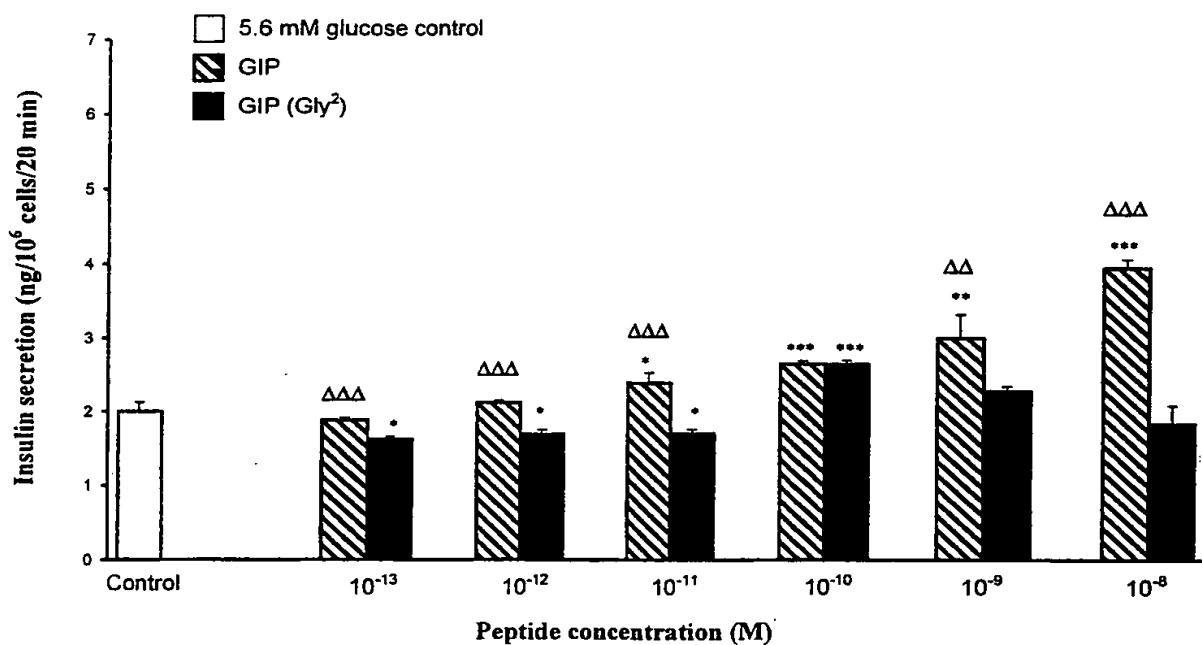
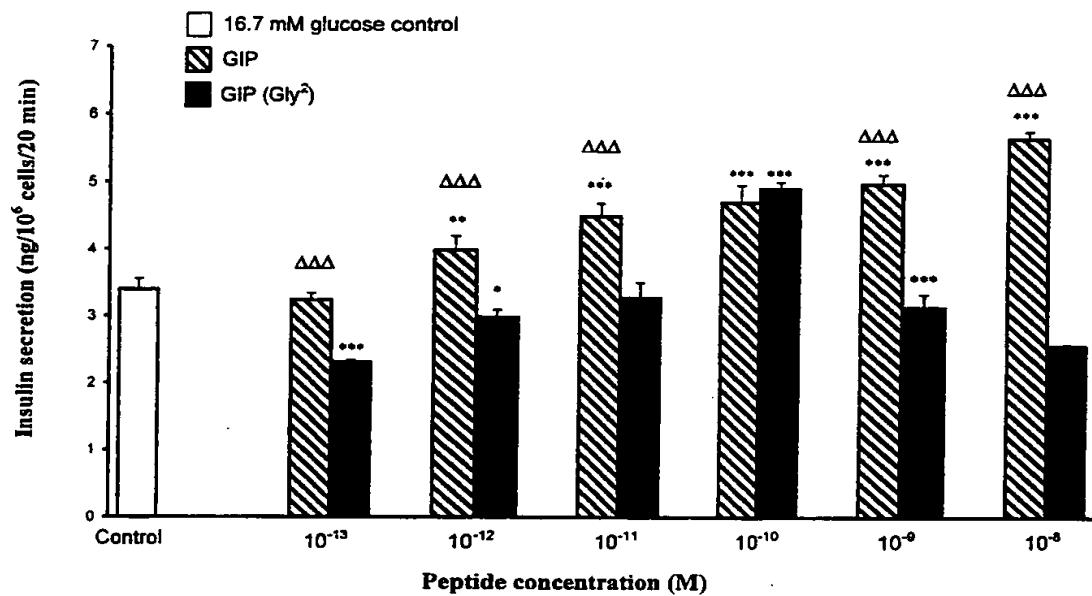


Fig. 27 Graph showing the effects of various concentrations of GIP and GIP (Gly^2) on insulin release from BRIN-BD11 cells incubated at 5.6 mM glucose



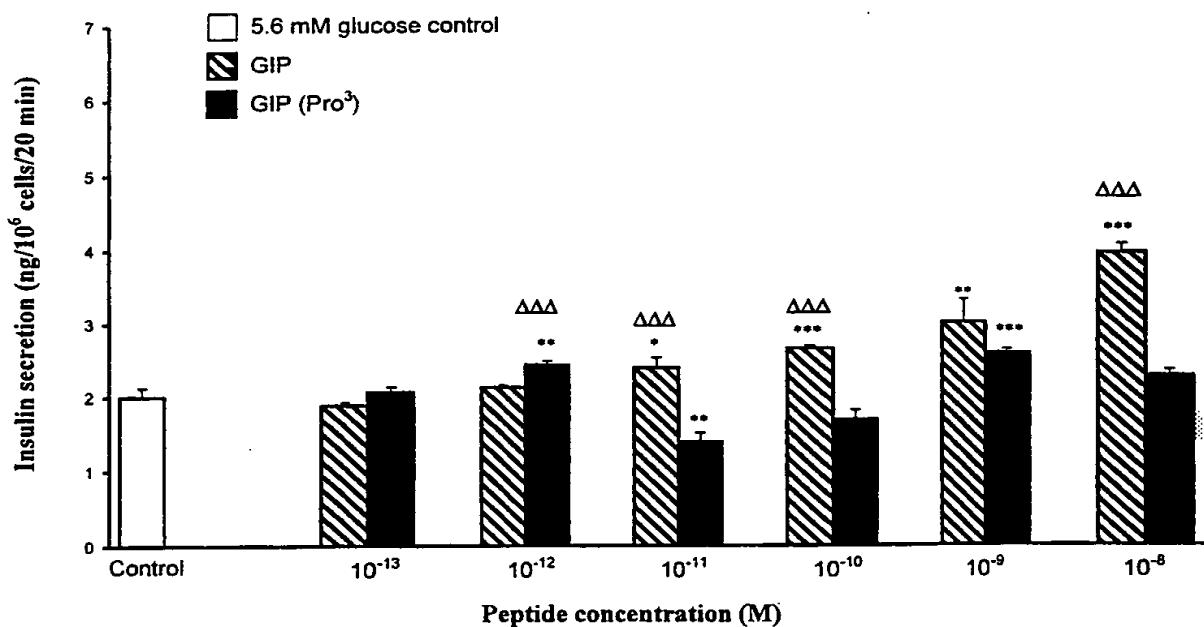
Values are means \pm S.E.M. for 12 separate observations. *P<0.05, **P<0.01, ***P<0.001 compared to control (5.6 mM glucose alone). ^P<0.05, ^AP<0.01, ^AAAP<0.001 compared to GIP (Gly^2) at the same concentration.

Fig. 28 Graph showing the effects of various concentrations of GIP and GIP (Gly^2) on insulin release from BRIN-BD11 cells incubated at 16.7 mM glucose



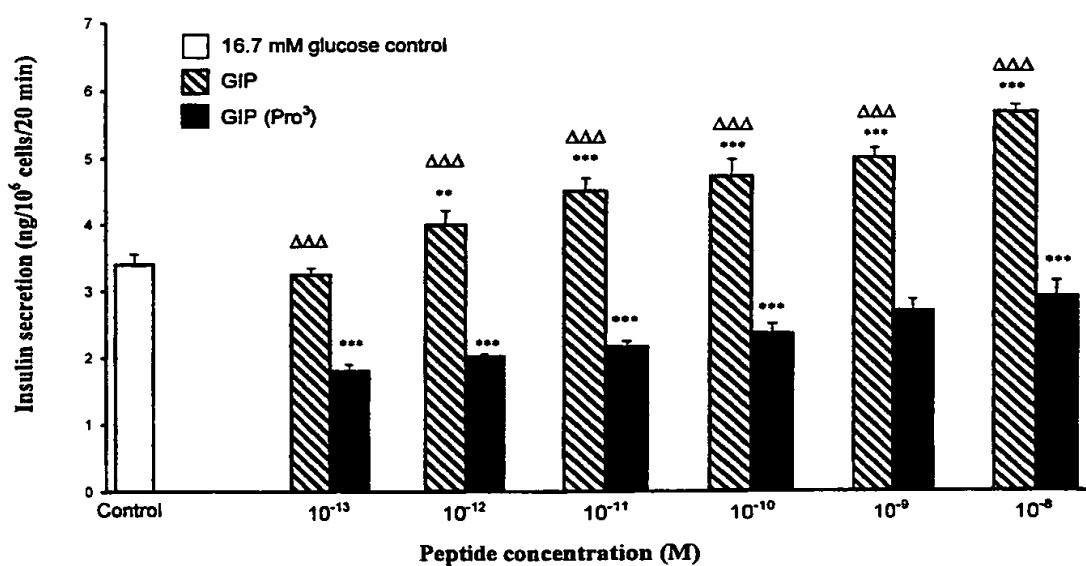
Values are means \pm S.E.M. for 12 separate observations. *P<0.05, **P<0.01, ***P<0.001 compared to control (16.7 mM glucose alone). ^P<0.05, ^AP<0.01, ^AAAP<0.001 compared to GIP (Gly^2) at the same concentration.

Fig. 29 Graph showing the effects of various concentrations of GIP and GIP (Pro³) on insulin release from BRIN-BD11 cells incubated at 5.6 mM glucose



Values are means \pm S.E.M. for 12 separate observations. *P<0.05, **P<0.01, ***P<0.001 compared to control (5.6mM glucose alone). ^P<0.05, ^*P<0.01, ^**P<0.001 compared to GIP (Pro³) at the same concentration.

Fig. 30 Graph showing the effects of various concentrations of GIP and GIP (Pro³) on insulin release from BRIN-BD11 cells incubated at 16.7 mM glucose



Values are means \pm S.E.M. for 12 separate observations. *P<0.05, **P<0.01, ***P<0.001 compared to control (16.7 mM glucose alone). ΔΔΔ P<0.001 compared to GIP (Pro³) at the same concentration.

PCT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION
(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Commissioner
US Department of Commerce
United States Patent and Trademark
Office, PCT
2011 South Clark Place Room
CP2/5C24
Arlington, VA 22202
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in its capacity as elected Office

Date of mailing (day/month/year) 08 November 2000 (08.11.00)	To:
International application No. PCT/GB00/01089	Applicant's or agent's file reference P23667A/RMC
International filing date (day/month/year) 29 March 2000 (29.03.00)	Priority date (day/month/year) 29 March 1999 (29.03.99)
Applicant O'HARTE, Finbarr, Paul, Mary et al	

1. The designated Office is hereby notified of its election made:

in the demand filed with the International Preliminary Examining Authority on:

21 September 2000 (21.09.00)

in a notice effecting later election filed with the International Bureau on:

2. The election was

was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Olivia TEFY
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PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

MURGITROYD & COMPANY
373 Scotland Street
Glasgow G5 8QA
ROYAUME-UNI

Date of mailing (day/month/year) 04 October 2001 (04.10.01)	
Applicant's or agent's file reference P23667A/RMC	IMPORTANT NOTIFICATION
International application No. PCT/GB00/01089	International filing date (day/month/year) 29 March 2000 (29.03.00)

1. The following indications appeared on record concerning:

the applicant the inventor the agent the common representative

Name and Address UNIVERSITY OF ULSTER Coleraine Co Londonderry BT52 1SA United Kingdom	State of Nationality GB	State of Residence GB
Telephone No.		
Facsimile No.		
Teleprinter No.		

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

the person the name the address the nationality the residence

Name and Address UUTECH LIMITED University House Cromore Road Coleraine Northern Ireland, BT52 1SA United Kingdom	State of Nationality GB	State of Residence GB
Telephone No.		
Facsimile No.		
Teleprinter No.		

3. Further observations, if necessary:

4. A copy of this notification has been sent to:	
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the receiving Office the designated Offices concerned
 the International Searching Authority the elected Offices concerned
 the International Preliminary Examining Authority other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Anman QIU Telephone No.: (41-22) 338.83.38
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